

GALACTURONOSYLTRANSFERASES, NUCLEIC ACIDS ENCODING SAME AND USES THEREFOR

CROSS REFERENCE TO RELATED APPLICATIONS

The present application claims benefit of United States Provisional Patent Application No. 60/445,539 filed February 6, 2003, which is incorporated in its entirety herein by reference to the extent not inconsistent herewith.

BACKGROUND

This invention relates to plant physiology, growth, development, defense and, in particular, to plant genes, termed galacturonosyltransferases (GALATs), nucleic acids encoding same and the uses therefor.

Pectins are the most complex polysaccharides in the plant cell wall. They comprise 30-40% of the primary wall of dicots and non-graminaceous monocots, and ~ 10% of the primary wall in the grass family. Pectins are a family of polysaccharides^{6,8,27} that include homogalacturonan (HGA) (Fig. 1), rhamnogalacturonan-I (RG-I) (Fig. 2) and rhamnogalacturonan II (RG-II) (Fig. 3) as well as xylogalacturonans (XGA)^{32,34,38} and apiogalacturonans.^{6,37} While the specific structure of each of these polysaccharides differs as shown in Figs. 1-3, they are grouped into one family since they appear to be linked to each other in the wall and they each contain α -D-galacturonic acid connected by a 1,4-linkage.

HGA is the most abundant pectic polysaccharide, accounting for ~55%-70% of pectin³⁹. HGA is a linear homopolymer of α 1,4-linked D-galactosyluronic acid that is partially methylesterified at the C6 carboxyl group and may be partially acetylated at O-2 and/or O-3⁸ (Fig. 1). Some plants also contain HGA that is substituted at the 2 or 3 position by D-apiofuranose, the so-called apiogalacturonans (AGA)^{36,37} and/or HGA that is substituted at the 3 position with D-xylose³²⁻³⁵, so-called xylogalacturonan (XGA). RG-II is a complex polysaccharide

that accounts for approximately 10-11% of pectin^{8,39}. RG-II has an HGA backbone with four structurally complex side chains attached to C-2 and/or C-3 of the GalA^{8,27} (Fig. 3). Rhamnogalacturonan I (RG-I) accounts for 20-35% of pectin³⁹ (Fig. 2). RG-I is a family of polysaccharides with an alternating [\rightarrow 4)- α -D-GalA-(1 \rightarrow 2)- α -L-
5 Rha-(1 \rightarrow] backbone in which roughly 20-80% of the rhamnoses are substituted by arabinan, galactan, or arabinogalactan side branches^{6,8,30}.

Pectins are believed to have multiple roles during plant growth, development, and in plant defense responses. For example, pectic
10 polysaccharides play essential roles in cell wall structure⁴³, cell adhesion⁴⁴ and cell signaling^{45,46}. Pectins also appear to mediate pollen tube growth⁴⁷ and to have roles during seed hydration^{48,49}, leaf abscission⁵⁰, water movement⁵¹, and fruit development^{47,8}. Oligosaccharides cleaved from pectin also serve as signals to induce plant defense responses^{52,53}. Studies of mutant plants with altered wall
15 pectin reveal that modifications of pectin structure leads to dwarfed plants⁴³, brittle leaves⁴⁴, reduced numbers of side shoots and flowers⁵⁴, malformed stomata⁴⁴ and reduced cell adhesion⁵⁵.

Although pectins appear to have multiple roles in plants, in no case has their
20 specific mechanism of action been determined. One way to directly test the biological roles of pectins, and to study their mechanisms of action, is to produce plants with specific alterations in pectin structure. This can be done by knocking out genes that encode the pectin biosynthetic enzymes. Such enzymes include the nucleotide-sugar biosynthetic enzymes and the glycosyltransferases that
25 synthesize the pectic polysaccharides. Each glycosyltransferase is expected to transfer a unique glycosyl residue in a specific linkage onto a specific polymeric/oligomeric acceptor. To date, only five^{56-59,136} of the more than 200 predicted wall biosynthetic glycosyltransferases have been functionally identified at the gene level (i.e. enzyme activity of the gene product proven), and none of these
30 have been shown to encode pectin biosynthetic enzymes.

Based on the known structure of pectin, at least 58 distinct glycosyl-, methyl- and acetyl-transferases are believed to be required to synthesize the family of

polymers known as pectin. As shown in the review by Mohnen, D. (2002) "Pectins and their Manipulation", G.B. Seymour *et al.*, Blackwell Publishing and CRC Press, Oxford, England, pp. 52-98, and Table I below, a minimum of 4-9 galacturonosyltransferases are predicted to be required for the synthesis of HGA, RG-I, RG-II and possibly for the synthesis of the modified forms of HGA known as XGA and AGA. The present invention relates to the identification of the first gene, *GALAT1*, encoding a galacturonosyltransferase and related genes thereto. The studies disclosed hereinbelow led the inventors to conclude that the gene *GALAT1* encodes the enzyme known as *UDP-GalA:Homogalacturonan α -1,4-Galacturonosyltransferase*.

Table I. List of galacturonosyltransferase activities predicted to be required for pectin biosynthesis⁹

Type of GalAT	Working ¹ Number	Parent polymer ²	Enzyme ³ Acceptor substrate Enzyme activity	Ref for Structure
D-GalAT	1	HGA	*GalA α 1 \rightarrow 4GalA α 1,4-GalAT	27
D-GalAT	2	RG-I	L-Rha α 1 \rightarrow 4GalA α 1,2-GalAT	27-29
D-GalAT	3	RG-II	L-Rha β 1 \rightarrow 3Apif α 1,2-GalAT	30,31
D-GalAT	4	RG-II	L-Rha β 1 \rightarrow 3Apif β 1,3GalAT	30,31
D-GalAT	5 ? ⁴	RG-I/HGA	GalA α 1 \rightarrow 2LRha α 1,4-GalAT	---
D-GalAT	6 ?	RG-II/HGA	GalA α 1 \rightarrow 4GalA α 1,4-GalAT	---
D-GalAT	7?	XGA	GalA α 1 \rightarrow 4(Xyl β 1 \rightarrow 3)GalA ⁵ α 1,4-GalAT	32-35
D-GalAT	8 ?	AGA	GalA α 1 \rightarrow 4(Apif β 1 \rightarrow 2)GalA α 1,4-GalAT	36,37
D-GalAT	9 ?	AGA	GalA α 1 \rightarrow 4(Apif β 1 \rightarrow 3)GalA α 1,4-GalAT	36,37

¹Numbers for different members of the same groups are given based on pectin structure and on the assumption that HGA is synthesized first, followed by RG-I and RG-II. The numbers were given⁹ to facilitate a comparison of the enzymes, but final numbering will likely correspond to the order in which the genes are identified.

²HGA: homogalacturonan; RG-I: Rhamnogalacturonan I; RG-II: Rhamnogalacturonan II; XGA: Xylogalacturonan; AGA; Apiogalacturonan.

³All sugars are D sugars and have pyranose rings unless otherwise indicated. Glycosyltransferases add to the glycosyl residue on the left* of the indicated acceptor.

⁴The ? means the designated GalAT may be required if a different GalAT in the list does not perform the designated function.

⁵Glycosyl residue in the parenthesis is branched off the first GalA.

Over the years, membrane-bound α 1-4galacturonosyltransferase (GalAT) activity has been identified and partially characterized in mung bean^{10,11}, tomato¹², turnip¹², sycamore¹³, tobacco suspension², radish roots⁵, enriched Golgi from pea⁷,

Azuki bean¹⁴, *Petunia*¹⁵, and *Arabidopsis* (see Table II). The pea GalAT was found to be localized to the Golgi⁷ with its catalytic site facing the lumenal side of the Golgi⁷. These results provided the first direct enzymatic evidence that the synthesis of HGA occurs in the Golgi. In *in vitro* reactions, GalAT adds [¹⁴C]GalA from UDP-[¹⁴C]GalA^{1,60} onto endogenous acceptors in microsomal membrane preparations to produce radiolabeled products of large molecular mass (i.e. ~105 kd in tobacco microsomal membranes² and ≥ 500 kd in pea Golgi⁷). The cleavage of up to 89% of the radiolabeled product into GalA, digalacturonic acid (diGalA) and trigalacturonic acid (triGalA) following exhaustive hydrolysis with a purified endopolygalacturonase confirmed that the product synthesized by tobacco GalAT was largely HGA. Thus, the crude enzyme catalyzes the reaction *in vitro*: UDP-GalAT + HGA(n) → HGA(n+1) + UDP. The product produced *in vitro* in tobacco microsomes was ~ 50% esterified² while the product produce in pea Golgi did not appear to be heavily esterified⁷. These results suggest that the degree of methyl esterification of newly synthesized HGA may be species specific and that methylesterification occurs after the synthesis of at least a short stretch of HGA. GalAT in detergent-permeabilized microsomes from azuki bean seedlings added [¹⁴C]GalA from UDP-[¹⁴C]GalA onto acid-soluble polygalacturonate (PGA) exogenous acceptors¹⁴. Treatment of the radiolabeled product with a purified fungal endopolygalacturonase yielded GalA and diGalA, confirming that the activity identified was a GalAT comparable to that studied in tobacco and pea. The azuki bean enzyme had a surprisingly high specific activity of 1300-2000 pmol mg⁻¹ min⁻¹, especially considering the large amount (3.1-4.1 nmol mg⁻¹ min⁻¹) of polygalacturonase activity that was also present in the microsomal preparations. As with the product made by tobacco, no evidence for the processive transfer of galactosyluronic acid residues onto the acceptor was obtained (see below).

Table II. Comparison of apparent catalytic constants and pH optimum of HGA- α 1,4-galacturonosyltransferases^{1,2}

Enzyme ²	Plant Source	Apparent K _m for UDP-GalA (μ M)	pH optimum	Vmax (pmol mg ⁻¹ min ⁻¹)	Ref
GalAT ¹	mung bean	1.7	6.0	~4700	¹⁰
GalAT	mung bean	n.d.	n.d.	n.d.	⁸¹
GalAT	pea	n.d. ⁵	6.0	n.d.	⁸²
GalAT	pea	n.d.	n.d.	n.d.	⁷
GalAT	sycamore	770	n.d.	?	¹³
GalAT	tobacco	8.9	7.8	150	²
GalAT (sol) ³	tobacco	37	6.3-7.8	290	³
GalAT (sol) ³	Petunia	170	7.0	480	¹⁵
GalAT (per) ⁴	Azuki bean	140	6.8-7.8	2700	¹⁴

¹Adapted from ref 6.² Unless indicated, all enzymes are measured in particulate preparations.³ (sol): detergent-solubilized enzyme.⁴ (per): detergent-permeabilized enzyme.⁵ n.d.: not determined.

GalAT can be solubilized from membranes with detergent³. Solubilized GalAT adds GalA onto the non-reducing end⁴ of exogenous HGA (oligogalacturonide; OGA) acceptors of a degree of polymerization of at least ten². The bulk of the HGA elongated *in vitro* by solubilized GalAT from tobacco membranes³, or detergent-permeabilized Golgi from pea⁷, at roughly equimolar UDP-GalA:acceptor concentrations is elongated by a single GalA residue. These results suggest that solubilized GalAT *in vitro* acts nonprocessively, (i.e. distributively). The apparent lack of *in vitro* processivity of GalAT was recently confirmed by Akita *et al.* who, using pyridylaminated oligogalacturonates as substrates and high concentrations of UDP-GalA, showed that although OGAs can be elongated in a "successive" fashion with up to 10 GalA residues by solubilized enzyme from petunia pollen¹⁵, the kinetics of this response suggest a distributive mode of action. We have two working hypotheses as to why GalAT *in vitro* does not appear to act processively. One hypothesis is that the solubilized enzyme or the enzyme in particulate preparations does not have the required factors, or is not present in the required complex, to act processively. An alternative hypothesis is that for a Golgi-localized enzyme that synthesizes a complex polymer in a confined

internal cellular compartment, such as GalAT, with sufficiently high concentrations of substrate, it would not necessarily be advantageous for the enzyme to act processively. In fact, the reaction velocity could be hindered under such conditions if the enzyme were processive⁶⁵.

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The apparent kinetic constants and pH optimum for the characterized GalATs are shown in Table II. We have performed additional kinetic studies in tobacco and radish that suggest that solubilized and membrane bound GalAT may have unusual apparent biphasic kinetics. We tested V_o for radish GalAT at 2 μ M to 10 80 mM UDP-GalA and obtained a biphasic curve (Fig. 4), suggesting that the kinetics of GalAT, at least in the membrane and soluble fractions, are complex. Comparable results were also obtained for the solubilized radish and tobacco enzyme. The initial V_o vs [UDP-GalA] curve was hyperbolic and appeared to reach an initial maximum V_o of ~ 300 pmol $\text{mg}^{-1} \text{min}^{-1}$ at ~ 1 mM UDP-GalA, confirming 15 previous results reported for tobacco^{2,3}. However, at ≥ 2 mM UDP-GalA there was a second hyperbolic increase in GalAT activity that reached a maximum of ~ 2 -4 nmol $\text{min}^{-1} \text{mg}^{-1}$ with ~ 20 mM UDP-GalA. In crude enzyme preparations it was not possible to determine the basis for the unusual kinetics. One possibility is that two GalATs were present, one with a low K_m and one with a high K_m . Another 20 possibility is that UDP-GalA is both a substrate and an allosteric regulator of GalAT. Alternatively, a more "trivial" explanation is that at low substrate concentrations the kinetics of GalAT were effected by a catabolic enzyme (e.g. a phosphodiesterase) in the enzyme preparation.

25 As a first step towards elucidating the role of galacturonosyltransferase (GALAT) in pectin synthesis, the inventors herein identified an *Arabidopsis* gene encoding alpha1,4- galacturonosyltransferase 1 (GALAT1). The database searches using the amino acid sequence of the GALAT1 identified fourteen additional GALAT family members and ten GALAT-like genes. The identification of 30 these genes and the availability of the sequence information allow the characterization of the enzyme, the use of these genes to produce mutated enzymes *in vivo* and *in vitro*, and transgenic plants producing modified pectins, and

studies of the role of a specific GalAT in pectin synthesis. The advantages of the present invention will become apparent in the following description.

SUMMARY OF THE INVENTION

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The present invention provides an isolated nucleic acid molecule encoding the polypeptide having galacturonosyltransferase (GalAT) activity. The *GALAT 1* disclosed herein represents the first functionally proven pectin biosynthetic glycosyltransferase gene isolated from plants. Also provided are additional 14 *GALAT* gene family members and 10 *GALAT*-like genes predicted to have galacturonosyltransferase activity. The identification and availability of the nucleic acid molecules as a member of the *GALAT* gene superfamily offer new opportunities to modulate pectin synthesis *in vivo* and *in vitro* by modulating the *GALAT* gene using various art-known recombinant DNA technology. For example, transgenic plants that produce modified pectins of desired properties can be generated by manipulating the gene encoding the GALAT protein i.e., mutating the gene including coding and non-coding sequences, silencing the gene by RNAi approach, or by administering a composition that would affect the GalAT activity in the plant. Since modified pectins are predicted to affect plant growth, development, and plant defense responses, the transgenic plants thus modified are expected to have improved agricultural value. The modified pectins can be isolated from such transgenic plants according to the art-known methods and serve as gelling and stabilizing agents of improved properties in the food, nutraceutical, and pharmaceutical industries.

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The inventors herein identified the first gene, *GALAT1*, which encodes a pectin biosynthetic enzyme by employing a partial purification-tandem mass spectrometry approach combined with a search of the *Arabidopsis* gene/protein database. Two genes, designated JS33 and JS36 herein, were identified as present only in the GalAT-containing fractions. As demonstrated hereinbelow, the expressed protein from the nucleic acid sequence of JS36 indeed exhibits the predicted GalAT enzymatic activity.

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A standard protein blast and a PSI Blast of the NCBI protein database using the GALAT1 (JS36) amino acid sequence revealed that *GALAT1* is a member of a 15 member *GALAT* gene family in *Arabidopsis*. The genes selected for this family have at least 30% amino acid identity and at least 50% amino acid similarity based on the PSI Blast. The database search using the *GALAT1* sequence further identified 10 *GALAT*-like genes as shown in Table IV. The genes disclosed herein, fifteen *GALAT* genes and ten *GALAT-like* genes thus represent the *GALAT* gene superfamily members.

The availability of the amino acid and nucleotide sequences of the *GALAT* gene superfamily members makes it possible to identify other *GALAT* homologs in other plants. The nucleotide and amino acid sequences of the *GALAT* genes can also be used to generate specific antibodies for the protein.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 shows the trimeric region of homogalacturonan (HGA). HGA is a linear homopolymer of alpha-1,4-linked galacturonic acid that may be methylesterified at C6 and acetylated at O2 or O3. Substituted galacturonans, such as RG-II and apiogalacturonan, have an HGA backbone.

Fig. 2 shows the representative structure of rhamnogalacturonan I (RG-I). RG-I has an alternating [\rightarrow 4)-alpha-D-GalpA-(1 \rightarrow 2)-alpha-L-Rhap-(1 \rightarrow) backbone in which roughly 20-80% of the rhamnoses are substituted by arabinans, galactans, or arabinogalactans.

Fig. 3 shows the representative structure for rhamnogalacturonan II (RG-II). RG-II has a backbone of 1,4-linked alpha-D-GalpA residues. GalA residues are also present in RG-II side chain A.

Fig. 4 illustrates the GalAT kinetics in radish microsomal membranes. Radish microsomal membranes (60-80 μ g protein) were incubated with 70 μ g of OGA (DP 7-23) and the indicated concentrations of UDP-GalA. Each reaction

contained a small concentration of UDP-[¹⁴C]GalA (2-3.6 μM) with larger amounts of nonradioactive UDP-GalA. The precipitated reaction products were measured by liquid scintillation counting. The data are the averages of duplicate samples from three separate experiments. The Y axis is specific activity (pmole min⁻¹mg⁻¹).

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Fig. 5 shows the outline of the strategy to identify the gene for GalAT. The sequenced *Arabidopsis* genome allowed the use of a function-based partial purification-mass spectrometry approach to identify the putative galacturonosyltransferase genes. The sample analyzed in each lane is as follows:

10 lane 1: homogenate, lane 2: total membranes, lane 3: solubilized proteins, lane 4: initial anion exchange purification step.

Figs. 6A and 6B show the results of RT-PCR experiments; 6A shows the results of JS33, JS36, and JS36L (a GalAT family gene with 63% identity to JS36) using *Arabidopsis* flower (F), root (R), stem (S), and leaf (L) RNA, and B shows the RT-PCR control using *Arabidopsis* actin gene in the same tissues.

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Fig. 7 is a schematic representation of the transmembrane spanning region and the conserved amino acids in the *Arabidopsis thaliana* GALAT gene family. The relative position of the strictly conserved residues among the members of the proposed GALAT family is numbered as for JS36 (i.e., GALAT1). The striped region from residues 22-44 represents the predicted transmembrane region.

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Fig. 8 demonstrates that recombinant JS36 (At3g61130) has galacturonosyltransferase (GalAT) activity. Human embryonic kidney cells (HEK293) were transiently transfected with the pEAK vector alone, or with pEAK vector containing the truncated versions of JS33 or JS36. Total media (1); protein immunoabsorbed from the medium using anti-HA epitope:Protein A Sepharose (2); and protein immunoabsorbed from the medium using anti-HA epitope:Protein G Sepharose (3) were tested for GalAT activity. Data are the average [¹⁴C]GalA incorporated into product from duplicate reactions from three separate experiments.

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Fig. 9 shows the relationship of the *Arabidopsis* GalAT superfamily including the GalAT family and the GalAT-like family. The Neighbor-Joining Tree is based on a sequence alignment generated by ClustalX.

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DETAILED DESCRIPTION OF THE INVENTION

In general the terms and phrases used herein have their art-recognized meaning, which can be found by reference to standard texts, journal references
10 and contexts known to those skilled in the art. The following definitions are provided to clarify their specific use in the context of the invention.

In the present application, the designation, "GALAT", is used to denote the gene for galacturonosyltransferase, "GALAT" is used to denote the protein encoded
15 by the gene, and "GalAT" is used to indicate galacturonosyltransferase enzyme activity.

The term, "polypeptide", is used herein interchangeably with "protein" to indicate a product encoded by a given nucleic acid.
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The terms, "identity" or "similarity" as used herein, are intended to indicate the degree of homology between the two or more nucleic acid or amino acid sequences. The degree of identity or similarity can be determined using any one of the computer programs that are well known in the art. The National Center for
25 Biotechnology Information (NCBI) website on the internet provides detailed description and references necessary for this subject. Also see Karlin and Altschul (1993) *Proc. Natl. Acad. Sci. USA* **90**:5873-5877; Altschul *et al.* (1997) *Nucl. Acids. Res.* **25**:3389-3402. In the present application, the percent amino acid identity and similarity among the GALAT gene family and GALAT-like gene family members
30 were carried out using the NCBI Pairwise Blast and Matrix Blosum62 using the GALAT1(JS 36) amino acid sequence.

A "corresponding" nucleic acid or amino acid or sequence of either, as used herein, is one present at a site in a GALAT molecule or fragment thereof that has
35 the same structure and/or function at a site in another GALAT molecule, although the nucleic acid or amino acid position may not be identical.

The term "gene" is used herein in the broadest context and includes a classical genomic gene consisting of transcriptional and/or translational regulatory sequences and/or a coding region and/or nontranslated sequences (i.e., introns, 5'- and 3'-untranslated sequences), or mRNA or cDNA corresponding to the coding regions (i.e., exons) and 5'- and 3'-untranslated sequences.

The meaning of a "homolog" as used herein is intended to indicate any gene or gene product which has a structural or functional similarity to the gene or gene product in point. For example, a new homolog of a given *GALAT* gene can be identified either by a database search using the amino acid or nucleic acid sequences of a given *GALAT* gene or by screening appropriate cDNA or genomic libraries according to the art-known methods.

An "expression vector" as used herein, generally refers to a nucleic acid molecule which is capable of expressing a protein or a nucleic acid molecule of interest in a host cell. Typically, such vectors comprise a promoter sequence (e.g. TATA box, CATTbox, enhancer etc) fused to a heterologous sequence (i.e., a nucleic acid of interest), sense or antisense strand, followed by a transcriptional termination sequence, a selectable marker, and other regulatory sequences necessary for transcription and translation of the nucleic acid of interest. A plant expressible promoter is a promoter comprising all the necessary so called regulatory sequences for transcription and translation of a gene of interest in plants. The linkage between the heterologous sequence and the regulatory sequences (e.g., promoter) is "in operable linkage" when a desired product can be made from the heterologous sequence under the control of the given regulatory sequences. An "expression vector" is often used interchangeably with an "expression construct" in this sense.

The term "transgenic plant" as used herein refers to a plant that has been transformed to contain a heterologous nucleic acid, i.e., a plant expression vector or construct for a desired phenotype. The transgenic plant is intended to include whole plant, plants parts (stems, roots, leaves etc.) or organs, plant cells, seeds, and progeny of same. The transgenic plant having modified pectin of the present application is one that has been generated by manipulating the gene encoding the *GALAT* protein. This can be achieved, for example, by mutating the gene, silencing

the gene by RNAi approach, or by knocking out the gene. The transgenic plants of the invention are predicted to have properties such as changes in organ and plant size, water transport properties, ease of removal of leaves and fruits via effects on abscission, pollen development and release, fruit ripening, root mucilage production, root growth, root cell cap production and separation, stem elongation, shoot growth, flower formation, tuber yield, defense responses against pathogens, and stomata opening⁸. Thus, the invention provides new means of improving plants of agricultural value. The "modified" pectins are those that exhibit structures and properties (e.g., gelling and stabilizing) different from those of the pectins naturally present in plants. Since galacturonic acid is a component of each of the pectic polysaccharides (i.e. HGA, RG-I, RG-II and XGA), a modification of the GalATs that add the specific GalAs into the specific polysaccharides is expected to modify the unique polymers. Such changes in pectin structure would affect multiple pectin properties including ionic interactions between HGA regions, gelation properties, dimer formation of RG-II molecules, length and degree of branching of RG-I, and side branch structure of RG-II. Such modifications are predicted to not only affect the biological function of pectin in plants, and the chemical and biological properties of pectin extracted and used by the food and cosmetic industries, but also properties that affect the use of pectin as a biopolymer for industrial processes, as a drug delivery polymer, and pectins of medicinal and nutraceutical properties in human and animal health.

The term "mutation" as used herein refers to a modification of the natural nucleotide sequence of a nucleic acid molecule made by deleting, substituting, or adding a nucleotide(s) in such a way that the protein encoded by the modified nucleic acid is altered structurally and functionally. The mutation in this sense includes those modifications of a given gene outside of the coding region.

The present invention provides polypeptides and nucleic acids encoding the polypeptides belonging to a family of the pectin biosynthetic enzyme, galacturonosyltransferase (GALAT). Pectins have been implicated in a broad range of plant growth phenomena including pollen tube growth⁴⁷, seed hydration⁴⁸⁻⁴⁹, leaf abscission⁵⁰, water movement¹²⁸, and fruit development⁸. In addition, pectic oligosaccharides serve as signals⁴⁵ during plant development⁴⁵ and induce plant

defense responses⁵²⁻⁵³. Mutant studies have shown that altered pectin structure leads to dwarfed plants⁴³, brittle leaves⁴⁴, reduced numbers of side shoots and flowers¹²⁹, and plants with reduced cell-cell adhesion^{130, 55}. Therefore, the present invention provides the molecular and biochemical tools needed to identify additional glycosyltransferases involved in branching of the backbones, and would allow the generation of plants with altered pectin structure. While the 25 genes disclosed herein represent only ~0.1% of the ~28,000 genes in *Arabidopsis*, they are some of the most difficult genes to identify and characterize because of a lack of commercially available acceptor substrates and activated glycosyl donor substrates.

The *GALAT1* gene has high sequence similarity to proteins expressed in other plants, thus using the sequences disclosed herein, a person of ordinary skill in the art can identify other pectin biosynthetic genes (i.e. homologs) in other plant species, including agriculturally important plants. Since pectin of very similar structure is present in the walls of all flowering plants and gymnosperms, the identification of functional pectin biosynthetic genes will greatly facilitate the engineering of plants with modified pectin and with altered growth characteristics, some of which are expected to yield plants of increased agronomical value. In addition, mutant plants with defined changes in pectin synthesis can allow the dissection of the biological role of each pectic component in plants. The pectin biosynthetic genes provide valuable tools for understanding mechanistically how pectin is synthesized. The glycosyltransferase-specific antibodies that can be generated using the sequences disclosed herein are also within the scope of the invention and allow the process of pectin assembly in the Golgi to be elucidated. A complete understanding of such a polysaccharide cellular trafficking process is unknown in any biological system.

Pectin is found in fruits and vegetables and is used as a gelling and stabilizing agent in the food industry. Pectin has been shown to have multiple beneficial effects on mammalian systems and on human health including the inhibition of cancer growth and metastasis, inhibition of cancer metastasis by binding of pectic oligosaccharides to cell surface receptors of cancer cells (US5834442, US5895784), immunomodulatory effects and stimulation of tumor

necrosis factor by macrophages (EP03983113), interaction with mucous cell lining of the duodenum and the prevention of ulcers (US4698229, US6024959); and anti-complementary activity¹²⁵. Many cancer cells have specific carbohydrate-binding protein molecules on their cell surfaces called galectins (galactoside-binding lectins). Galectins aid in cellular interactions by binding to beta-galactose linked molecules on neighboring cancer cells. Galectin-3 is a multifunctional lectin that is involved in tumor cell adhesion, metastasis and cancer progression. Blocking galectin-3 expression in malignant human breast, papillary and tongue carcinoma cells led to reversion of the transformed phenotype and suppression of tumor growth in nude mice¹¹⁷⁻¹¹⁹. A pH-modified citrus pectin is suggested to block binding of galectins and inhibit tumor cells adhesion. Pienta *et al.*¹²⁷ showed that feeding of pH-modified pectin to rats caused a reduction in metastasis of prostate cancer. Similarly, oral administration of pectin to mice carrying colon tumors, reduced tumor size compared to control animals¹¹⁴, reduced metastatic colonization of B16-F1 melanoma in the lung¹²⁰⁻¹²¹ and reduced human breast and colon carcinoma growth, angiogenesis, and metastasis¹²⁵. When prostate cancer patients were fed pH-modified citrus pectin, a 30% lengthening in prostate specific antigen (PSA) doubling time was observed in 57% of the patients¹²². As progression of prostate cancer is evaluated based on the time that it takes for the PSA to double, the above observations suggested that pectins may reduce tumor size. It has also been shown that fruit-derived pectins inhibit the interaction of fibroblast growth factor 1 (FGF1) to its receptor (FGFR1)¹²³. Defects in the FGF signal transduction system are known to disturb cellular regulatory processes resulting in cancer, cardiovascular disease and diabetes mellitus. The availability of the gene(s) encoding galacturonosyltransferase allows the modification of neutraceutical or pharmaceutical pectins to provide pectins with novel cell and molecule binding activities and thus, with novel and specified anticancer and other physiological activities.

In order to identify a gene(s) involved in pectin biosynthesis, the inventors used a partial purification-tandem mass spectrometry approach to identify putative *GALAT* genes from *Arabidopsis* (see Fig. 5 for strategy). GalAT from *Arabidopsis* was partially purified from detergent-solubilized enzyme by sequential passage over two or more of the following resins: cation exchange resin SP-Sepharose,

reactive green 19 resin, reactive blue 72 resin, reactive yellow 3 resin, and UDP-agarose. Proteins obtained from selected fractions from these columns were treated with trypsin to generate peptides, and the amino acid sequence of the peptides identified by liquid chromatography-tandem mass spectrometry. The amino sequence thus generated was used to screen the *Arabidopsis* gene/protein database. Thirty unique proteins were solely identified in the GalAT-containing fractions (i.e. not present in fractions not containing GalAT activity). Among the 30 unique proteins that co-purified with GalAT activity, two proteins (designated JS33 and JS36) were initially identified as *Arabidopsis* putative GALAT proteins/genes based on their having at least one predicted transmembrane domain and since they contained a predicted glycosyltransferase domain (see CAZy database; <http://afmb.cnrs-mrs.fr/CAZY/index.html>).

These two genes, along with another *Arabidopsis* gene with high sequence similarity to JS36 (designated JS36L for JS36-like) (see below) were either cloned by RT-PCR (JS36) using mRNA from *Arabidopsis* flower and stem tissue, or a cDNA clone was obtained from the Arabidopsis Biological Resource Center (JS33 and JS36L). The proteins encoded by these genes each have a predicted single transmembrane domain (Table III). The genes were truncated to remove their N-terminal region including all or most of the predicted transmembrane domain (see Table III), and the truncated genes were inserted into a mammalian expression vector pEAK10 (Edge BioSystems as modified by Kelley Moremen lab, CCRC) containing an N-terminal heterologous signal sequence (targeting the protein for secretion into the medium), a polyhistidine (HIS) tag, and two influenza hemagglutinin (HA) epitopes (useful for immunoabsorption).

Table III. Predicted characteristics of JS36, JS33 and JS36L proteins. Predictions were made using information from the NCBI database and the SOSUI (Classic & Membrane Prediction program) at BCM Search Launcher site (<http://searchlauncher.bcm.tmc.edu/seq-search/struct-predict.html>).

Gene	NCBI protein ID	# amino acids	MW (kd)	pI	Predicted transmembrane domain	Truncated protein
At3g61130 (JS36)	NP_191672	673	77.4	9.95	^N 22-44 ^C	^N 42-673 ^C
At2g38650 (JS33)	NP_565893	619	69.7	8.63	^N 23-45 ^C	^N 44-619 ^C
At5g47780 (JS36-like)	NP_568688	616	71.1	9.26	^N 6-22 ^C	^N 26-616 ^C

The truncated forms of JS33, JS36 and JS36L, and the vector alone, were transiently expressed in human embryonic kidney cells (HEK293 cells) for 46 hours. Since the translational fusion proteins constructed contained two copies of the HA epitope, the culture medium was collected and a portion was treated with a mouse anti-HA IgG1 bound either to Protein A Sepharose or Protein G Sepharose. The immunoadsorbed protein was assayed for GalAT activity using UDP-[¹⁴C]GalA and a mixture of OGA acceptors. Figure 8 shows that the JS36 construct expressed a protein exhibiting GalAT activity. These studies establish that JS36 is a GalAT and thus we designated the gene *GALAT1*.

As mentioned above, analysis of the amino acid sequence of GALAT1 shows that the expressed protein contains one transmembrane domain. This is in agreement with the GalAT activity being membrane bound in all species tested (see Mohnen *et al.* (2002)⁹. Furthermore, the predicted topology of GALAT1 is that of a type-II membrane protein, in agreement with our previous determination that the catalytic site of pea GalAT lies in the lumen of the Golgi. Type-II membrane proteins have a short N-terminal cytosolic tail, a transmembrane region, a stem region, and a C-terminal catalytic domain¹⁶.

GALAT1 is a member of the Glycosyltransferase Family 8 in the CAZy database [database of putative and proven carbohydrate modifying enzymes that currently contains 61 different proposed glycosyltransferase families (<http://afmb.cnrs-mrs.fr/CAZY/index.html>)^{66,67}]. The presence of GALAT1 in Family 8 is in agreement with our demonstrated activity of GALAT1 as an α 1,4-galacturonosyltransferase, since Family 8 is a family of proposed retaining glycosyltransferases and GALAT1 is a retaining enzyme, i.e., the α -configuration in the substrate UDP- α -GalA is retained in the product α 1,4-linked-galacturononan (HGA).

GALAT is expressed in multiple *Arabidopsis* tissues at multiple times during development. We base this on our RT-PCR analysis of RNA from *Arabidopsis* flower, root, stem and leaf tissue (Figs. 6A and 6B) showing that GALAT1 is expressed in all these tissues, and based on the 18 EST entries for this gene in the TAIR database (<http://www.arabidopsis.org/>) indicating that GALAT1 is expressed in developing seed, green siliques, roots and above ground organs.

Identification of the GALAT1 Gene Family

A standard protein blast and a PSI Blast of the NCBI protein database using the GALAT1 (JS36) amino acid sequence reveal that *GALAT1* is a member of a at least 15 member *GALAT* gene family in *Arabidopsis* (see Table IV). The genes selected for this family have at least 30% amino acid identity and at least 50% amino acid similarity based on the PSI Blast. We further compared these genes along their entire coding sequences with JS36 using a Pairwise BLAST (Table IV) and show that this family of genes has at least 34% identity and at least 52% similarity to JS36 in the portion of the genes C-terminal to the membrane spanning domain. This identity is comparable to the 37-54% identity shared among the proposed ten member *Arabidopsis* fucosyltransferase gene family (AtFU1-10)⁷¹.

Mutant studies provide further evidence that the GalAT family encodes GalATs involved in pectin synthesis. We recently used seed received from *Arabidopsis* T-DNA mutant collection (SIGnAL; <http://signal.salk.edu/cgi-bin/tdnaexpress>) to identify and generate six homozygous *Arabidopsis* GalAT family T-DNA insert mutant lines of several members of the GalAT family. We found that one GalAT family gene At1g06780, when mutated, produces leaves with cell walls that contain reduced amounts of galacturonic acid. Specifically, analysis of walls from homozygous mutant line 073484 revealed that the walls had an 18% reduction in GalA and a concomitant increase in glucose. None of the other sugars changed. Of the three available At1g06780 T-DNA insert lines, no homozygous seed was recovered from mutants where the T-DNA was inserted into an exon. Rather, seed recovered from such lines had a reduced germination rate. In line 073484, however, the T-DNA is inserted in the 5'-UTR, suggesting that it may have a leaky phenotype. The results are consistent with gene At1g06780 encoding a GalAT and with the identification of the gene family as a GalAT gene family. The GalA content of the walls of another *Arabidopsis* mutant (*Quasimodo*) is reduced by 25% and these plants exhibit decreased cell adhesion⁵⁵, characteristics consistent with the *Quasimodo* gene encoding a GalAT. *Quasimodo* has 53% amino acid identity and 72% similarity to GALAT1 and the gene affected in *Quasimodo* (At3g25140) is a member of our proposed GalAT family. There is, however, at present no direct enzymatic evidence that the protein encoded by *Quasimodo* is a functional GalAT.

The conserved amino acids in the *GALAT* gene family are shown in Fig. 7. Glycosyltransferases are expected to contain one or more carboxylates at the catalytic site. At least one of the carboxylates is expected to coordinate a divalent cation associated with the nucleotide-sugar. In many glycosyltransferases the metal coordination involves two carboxylates that are often present as DDx, xDD, or DDD (the so-called "D(x) D" motif)⁷².

A PSI Blast against *GALAT1* gene (JS36) further identified 10 genes that have high sequence identity (23-29%) and similarity (41-51%) to *GALAT1* and form a tight cluster of highly similar genes (55-66% identity/67-77% similarity). A Neighbor Joining Tree of our proposed *Arabidopsis* GalAT Superfamily (i.e. the proposed *GALAT* family and the *GALAT*-Like family), based on a sequence alignment generated by ClustalX¹²⁸, is shown in Fig. 9. The 10 *GALAT*-like genes are all significantly smaller, lacking ~200 amino acids in comparison with the *GALAT* family. Nonetheless, they appear to be targeted to the secretory pathway based on annotation of the genes at the *Arabidopsis* Information Resources. All 10 genes appear to be expressed in *Arabidopsis*, since they are represented by one or more ESTs in the *Arabidopsis* EST collection. The *GALAT*-like genes also contain some of the same conserved residues as the GalAT family, namely D-D-----D---L (the predicted "D(x) D" motif) and L-----F-----W---GLG-----H---G---KPW. We group the 10 *GALAT*-like genes into a family that encode GalATs directly involved in pectin synthesis or GalATs with, as yet, unidentified glycosylating function.

Table IV. Pairwise sequence alignment between JS36 and the other members of proposed GALAT gene family. The alignment was done using the NCBI Pairwise BLAST and Matrix Blosum62. The % amino acid identity and similarity are shown. In all cases the alignment compares the bulk of the C-terminal portion of the proteins on the carboxy-terminal side of the transmembrane region.

Gene	NCBI protein ID	EMBL protein #	% Identity (#aa identical/#aa)	% Similar amino acids (aa/aa)
GaIAT-Family				
***At3g61130 (GALAT1; JS36)	NP_191672	Q9LE59	100% (673/673)	100% (673/673)
At5g47780 (JS36-like)	NP_568688	Q93ZX7	63% (290/458)	81% (374/458)
At2g46480	NP_182171		61% (297/485)	75% (365/485)
At4g38270	NP_195540		55% (344/620)	73% (459/620)
At3g25140 (Quasimodo)	NP_189150	Q9LSG3	53% (241/450)	72% (330/450)
At1g18580	AAK93644		48% (226/469)	67% (317/469)
At3g02350	NP_566170	Q9FWA4	47% (247/521)	66% (350/521)
At2g20810	NP_565485	Q93VL7	46% (215/462)	68% (320/462)
At1g06780	NP_563771	Q9M9Y5	44% (204/461)	63% (296/461)
At2g30575	NP_850150		43% (203/463)	65% (309/463)
At3g01040	NP_186753	Q9MAB8	42% (189/447)	61% (227/447)
At5g15470	NP_197051	Q9LF35	42% (189/443)	61% (274/443)
At5g54690	NP_200280	Q9FH36	38% (169/436)	60% (265/436)
At2g38650 (JS33)	NP_565893	Q949N9	36% (171/475)	60% (286/475)
At3g58790	NP_191438	Q9LXS3	34% (160/458)	52% (247/458)
GaIAT-Like Family				
At1g02720	NP_171772		26 (85/316)	44 (143/316)
At1g13250	NP_563925	Q9FX71	23 (86/359)	41 (154/359)
At1g19300	NP_564077	Q9LN68	29 (58/198)	49 (98/198)
At1g24170	NP_173827	O48684	23 (75/322)	41 (136/322)
At1g70090	NP_564983	O04536	27 (64/233)	48 (115/233)
At3g06260	NP_187277	Q9M8J2	29 (52/179)	51 (92/179)
At3g28340	NP_189474	Q9LHD2	28 (56/194)	52 (104/194)
At3g50760	NP_190645	Q9S7G2	24 (76/308)	43 (137/308)
At3g62660	NP_191825	Q9LZJ9	29 (56/191)	51 (99/191)
At4g02130	NP_192122		29 (58/197)	51 (103/197)

The expression of the *GALAT1* gene in transiently transfected mammalian cells as demonstrated herein now allows the production of stably transformed cell lines that produce *GALAT1* and experiments aimed at characterizing the mechanism of the enzyme and at determining the role of *GalAT1* in pectin synthesis. Specifically, the substrate specificity of *GalAT1* will indicate whether it catalyzes only HGA synthesis, or also plays a role in RG-I and RG-II synthesis. Characterization of the kinetics of *GalAT1* can clarify whether or not UDP-GalA is both a substrate and an allosteric regulator of the enzyme. Characterization of the mutated *GalA1* enzyme can provide information regarding amino acids important in catalysis and substrate binding. The subcellular location of *GALAT1* will provide the first framework for where, within the Golgi and plant endomembrane system the complex series of pectin biosynthetic reactions occur. The invention can further be used to generate transgenic plants with modified pectin, which can provide information regarding the role of *GALAT1* in pectin synthesis, provide novel biosynthesis acceptors, and provide information about the role of pectin in plant growth and development. This biosynthesis framework allows further identification of *GALAT1* binding proteins that would be putative pectin biosynthesis complex members. The results of these studies can serve as the foundation for a full *in vitro* reconstitution of functional pectin synthesis complexes.

GALAT1 has high sequence similarity to 14 other *Arabidopsis* proteins as shown in Table IV and to proteins expressed in other plants. Possible *GALAT1* homologs in other plants are a 68 kd protein expressed in *Cicer arietinum* (chickpea) epicotyls (76% amino acid identity; 87% similarity), a hypothetical protein from *Oryza sativa* (japonica) (59% identify; 75% similarity) and a protein from *Populus alba* (49% identity; 72% similarity). Thus, the results from the study of *GALAT1* in *Arabidopsis* can be extended to other plants, including those of high agricultural value.

Heterologous expression of *GALAT1*

As described above, the media from human embryonic kidney (HEK293) cells transiently infected with recombinant expression vector bearing truncated *GALAT1* expressed *GALAT1*. Whereas transient expression allowed the

expression of sufficient GALAT to measure GalAT activity, additional expression strategies can be readily devised to produce large quantities of GALAT1 required for further characterization of the enzyme and for antibody production. Since the transiently expressed N-terminal epitope-tagged GALAT1 expressed in mammalian cells was active, one strategy is to produce stably transfected clonal HEK293 lines⁷⁵ expressing the same protein. The alternative strategy is to express the full length and N-terminal truncated forms of GALAT1 in the fungal expression system *Pichia pastoris*. These systems were chosen since we and others⁵⁶⁻⁵⁸ have successfully used them to express plant glycosyltransferases.

For expression in *P. pastoris*, cDNA encoding the entire, and the truncated soluble forms of GALAT can be generated by PCR using gene/vector specific primers. The PCR products are then subcloned into appropriate *Pichia* expression vectors (Invitrogen, Carlsbad, CA) in which the cDNA is inserted downstream from an alcohol oxidase (AOX1) promoter. We have made full length coding sequence constructs for expression in the *Pichia* vector pPIC 3.5. This vector does not contain an epitope tag. One can easily make epitope tagged GALAT1 constructs in the *Pichia* vectors pPICz and pPICz α (Invitrogen) and determine whether functional C-terminal epitope-tagged constructs that do not affect GalAT activity can be recovered. Several studies have demonstrated success of the *Pichia* system⁷⁶⁻⁸². Once a high-GALAT1-producing line is recovered, production of large amounts of protein can be carried out in fermentors or spinner flasks.

Characterization of Expressed GALAT1

To begin to address how HGA is synthesized, the kinetics, substrate specificity, and structure of the purified recombinantly expressed GALAT1 can be determined and compared to the solubilized membrane-bound *Arabidopsis* GALAT purified by immunoadsorption using the polyclonal-antiGALAT1 (see below). Although the characteristics of GalAT1 are consistent with the enzyme being the/a catalytic subunit of the HGA synthase, GALAT1 could be a GalAT involved in RG-II or RG-I synthesis. For example, GalAT could represent an RG-I:GalAT that initially elongates HGA by a single GalA and then waits for a required NDP-Rha to start RG-I backbone synthesis. The kinetics of purified and recombinantly expressed GALAT1 for UDP-GalA and a size range of homogalacturonan and pectin

acceptors can be determined. The effect of other nucleotide-sugars and oligosaccharide substrates on GalAT can also be tested to identify activators and inhibitors.

5 The expressed full length and truncated enzymes can be assayed in a reaction buffer in the presence, and absence, respectively, of Triton X-100. The kinetics of the enzyme for UDP-GalA can be carried out in a total of 1 μ M to 80 mM UDP-GalA + UDP-[14 C]GalA. We routinely synthesize UDP-[14 C]GalA either by the 4-epimerization of UDP-[14 C]GlcA¹ or oxidation of UDP-[14 C]Gal⁸⁴ since UDP-
10 [14 C]GalA is not commercially available. The effect of different acceptors on GALAT1 activity can be conducted using 100 μ M UDP-GalA and 0.1-100 μ g acceptor/ 30 μ l reaction. The acceptors to be tested include HGA oligosaccharides (oligogalacturonides) of degrees of polymerization ranging from 2-16, polygalacturonic acid, commercially available citrus pectin of ~30, 60 and 90%
15 esterification, RG-I and RG-II. The products made using the different acceptors can be characterized^{2,3}. If RG-I is shown to serve as an acceptor, RG-I backbone fragments that have a GalA or a Rha at the non-reducing end can be used to determine acceptor specificity. The acceptors can be tested using multiple assays including the precipitation assay² and a filter assay⁶³. The enzymes can also be
20 tested for the effect of pH, temperature, reducing agents, divalent cations and salts on enzyme activity and product structure.

Characteristics of the recombinant truncated GALAT1 can be compared to the GALAT1 solubilized from *Arabidopsis* membranes by immunoadsorption of the
25 solubilized GALAT1 using anti-GALAT1 antibody (see section below) bound to Protein A or G Sepharose, or by coupling the anti-GALAT1 antibodies to 3M-Emphaze resin⁸⁶ and using the resin used to purify GALAT1 from solubilized *Arabidopsis* enzyme. If the characteristics of the immunoadsorbed *Arabidopsis* GALAT1 are different from those of the recombinant truncated GALAT1, the
30 immunoadsorbed GALAT1 can be analyzed by LC tandem mass spectrometry to determine if additional proteins are immunoadsorbed with the *Arabidopsis* solubilized GALAT1 that may have modified the activity (e.g. a heteromeric complex).

The recombinant GALAT1 and the GALAT1 immunoadsorbed-from *Arabidopsis* solubilized membranes can also be treated with N-glycanase to determine if they are N-glycosylated. To determine if they are O-glycosylated, the proteins can be exhaustively treated with N-glycanase, the released oligosaccharides removed, and the resulting protein analyzed by TMS methylation analysis to determine the glycosyl residue composition of any carbohydrates still attached to the protein. Any oligosaccharide released by the N-glycanase treatment can also be analyzed by TMS methylation. The results of these experiments would indicate whether the native *Arabidopsis* GalAT is glycosylated and whether the recombinant forms have the same or different glycosylation pattern. Changes in glycosylation could affect GalAT1 enzyme activity and/or substrate binding. GALAT1 is predicted to have 5 or 6 N-glycosylation sites (NetNGlyc 1.0 Prediction; <http://www.expasy.org/sitemap.html>).

As mentioned above, we have found that membrane-bound and solubilized GalAT activity in tobacco and radish has unusual apparent biphasic kinetics. Thus, we are particularly interested in determining if the expressed GALAT1 shows the same kinetics, including possible allosteric regulation by UDP-GalA. One can test for possible multimeric structure by determining the mass of the enzyme by size exclusion chromatography and comparing these with the mass obtained by SDS-PAGE. The possibility that GALAT1 exists as a heteromultimer can be tested by mixing expressed recombinant GALAT1 with solubilized *Arabidopsis* enzymes and immunoadsorbing GALAT1 and proteins bound to it using either an anti-GALAT1 antibody or an anti-HA epitope antibody (see previous section).

Production of a series of mutated GALAT1 proteins by site-directed mutagenesis

As discussed above, there are 45 conserved amino acids in GALAT1 among the 15 members of the *GALAT* family. To determine the role of these residues in substrate/acceptor binding and/or catalysis, each amino acid is systematically mutated using site-directed mutagenesis. The effect of these mutations on GALAT1 specific activity, and where warranted, on K_m , V_{max} , and acceptor specificity (i.e. OGA, RG-I and RG-II) and product size (i.e. enzyme processivity) is determined.

Production and use of antibodies

Anti-GalAT antibodies are necessary for the immunocytochemistry experiments, to immunopurify solubilized GALAT1 from *Arabidopsis*, and to select proteins that potentially bind to GALAT1 and may function in pectin biosynthetic enzyme complexes. A skilled artisan can generate anti-GalAT antibodies using the nucleic acid or amino acid sequences disclosed herein. This can be accomplished by employing the heterologously expressed truncated or full-length GALAT1. Alternatively, a small peptide derived from the GALAT1 sequence can be synthesized and used to generate anti-GALAT1 antibodies. One can generate either polyclonal or monoclonal antibodies. Such antibodies are useful for a range of types of experiments, including subcellular immunocytochemistry, immunoprecipitation/adsorption, and enzyme activity inhibition studies. Monoclonal or polyclonal antibodies, specifically reacting with a protein of interest can be made by methods well known in the art. See, e.g., Harlow and Lane (1988) *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratories; Goding (1996) *Monoclonal Antibodies: Principles and Practice*, 3rd ed., Academic Press, San Diego, CA, and Ausubel et al. (1993) *Current Protocols in Molecular Biology*, Wiley Interscience/Greene Publishing, New York, NY.

Subcellular localization of GALAT1

All available data, including the localization of the catalytic domain of GalAT in the Golgi lumen⁷, suggest that pectin is synthesized in the Golgi and transferred via vesicles to the wall. However, it is not known how the different glycosyltransferases function to make specific pectin structures. We predict that different glycosyltransferases are localized in a sequential manner to different cisternae of the Golgi^{22,91} in an order indicative of the order in which pectin is synthesized as it moves from the cis, through the medial and to the trans Golgi. Evidence from both animal^{92,93} and plants⁹⁴ suggests that, either individually or in combination, the transmembrane domain (i.e. the bilayer thickness model⁹⁵), the N- or C-terminal sequences flanking the transmembrane domain, and/or the luminal domain (i.e. the 'kin recognition model'⁹⁶) contribute to localization of proteins within the Golgi system. The anti-GalAT antibodies generated as described above can be used to determine the subcellular localization of GALAT1 within the Golgi in order

to provide additional information on the role of GalAT1 in pectin synthesis. For example, a location of GALAT1 in the cis and medial Golgi cisternae would be consistent with a function of GALAT1 in HGA synthesis, while a localization primarily in the late medial or trans Golgi would be more suggestive of a role in RG-I or possible RG-II synthesis. It should be noted that such subcompartment localization studies, while important and novel for the pectin biosynthetic enzymes, are also novel in any species since the "precise location of only a small number of the glycosyltransferase proteins within the Golgi apparatus have been determined"⁹³. Anti-GALAT1 antibodies can be used to identify where in the Golgi GALAT1 is localized by, for example, immunogold label of thin sections from *Arabidopsis*^{97, 91, 98, 99} including both developing *Arabidopsis* seedlings and growing suspension cultures which have cells actively making wall.

Use of mutants and RNAi to generate and characterize GALAT1 and GalAT gene Superfamily knockouts.

Double-stranded RNA-mediated interference (RNAi) is a method to study the function of genes in plants¹⁰⁰. Transgenic plants harboring an RNAi construct often have reduced expression of the gene-specific mRNA. The resulting plants may display either complete gene silencing, thus having a knockout phenotype, or a partial "knockout" phenotype due to 'leaky' expression. The RNAi approach should allow the suppression of *GALAT1* expression and a reduction or loss of GALAT1. This enables one to elucidate the function of GALAT in pectin synthesis and in the plant. Simultaneously, the sequence-indexed T-DNA insertion mutants listed in the Salk Institute Genomic Analysis Laboratory (SIGnAL) *Arabidopsis* T-DNA mutant collection (<http://signal.salk.edu/cgi-bin/tdnaexpress>) can be monitored to determine if any T-DNA insert lines for GALAT become available. If so, the seed can be obtained and the mutants generated therefrom can be characterized (as described above).

The putative pectin biosynthesis mutants can aid in the identification of gene function in two ways. The visible phenotypes of the mutants can provide information on the biological function of the gene (if there is no redundancy in gene function) by demonstrating when during growth and development the particular

gene product is needed (as shown above). Structural analysis of the pectin in the mutant walls can provide information about the specific enzyme activity of the gene in pectin synthesis (as shown above).

5 Of particular importance regarding pectin synthesis, the cell walls are isolated and analyzed for glycosyl residue composition (see above) and linkage to provide information about the possible role of GALAT1 in pectin synthesis.

Identification of the members of HGA biosynthetic complexes.

10 There is growing evidence that glycoconjugates are synthesized by complexes of glycosyltransferases and other types of proteins¹⁰². For example, ganglioside synthesis occurs via a tightly regulated formation of multiple glycosyltransferase complexes¹⁰². Thus, any protein members of HGA biosynthetic
15 complexes can be isolated by immunoadsorbing such proteins bound to GALAT1 using anti-GALAT1 antibodies or anti-HA epitope antibodies. The immunoadsorbed proteins can be identified by SDS-PAGE, removed from the gel, and their amino acid sequence determined by LC-tandem mass spectrometry. The amino acid sequences thus obtained can then be used to search the available
20 protein databases for their identities.

Characterization of mutant phenotypes and bulking up of seed.

A person of ordinary skill in the art can use mutant seeds to probe gene
25 function. For example, the initial mutant seed (often a segregating T3 line, see http://signal.salk.edu/tdna_FAQs.html) can be grown and selfed to increase the seed stock (T4). Multiple plants from T4 seed can be grown and the presence of, for example) the T-DNA insert determined by PCR of plant genomic DNA using a T-DNA primer and a gene specific primer. The same DNA can be analyzed with gene
30 specific primers that should span the T-DNA insertion site. These analyses should indicate whether the given plant contains a T-DNA insert and if so, whether it is homozygous or heterozygous for the mutation. If necessary, Southern blotting and hybridization with the specific genes can be used to determine if the gene contains the expected T-DNA insert. Seed homozygous for the T-DNA insertion (when not
35 lethal) or heterozygous (when no viable TDNA homozygous plants are obtained) can be selfed to amplify the seed and, for heterozygous plants, to test for

segregation of any phenotype or T-DNA insert. Plants can be scored as heterozygous or homozygous by PCR analysis of the T-DNA insert and by any visible phenotype. Homozygous or heterozygous plants can be used for growth phenotype and cell wall analysis. The seed can also be crossed with wild type Columbia and then selfed to eliminate the possibility that the lines contain an unexpected mutation or additional T-DNA insert(s).

Growth Phenotype analysis

Several growth parameters of the mutant and wild type plants are recorded to yield a general phenotypic characterization of the mutant plants.¹³⁴

Analysis of Cell Walls

Homozygous or heterozygous plants are grown and analyzed for wall composition and linkage. Cell walls can, for example, be prepared as alcohol insoluble residues (AIRs) from WT and (homozygous) mutant *Arabidopsis* plant tissues¹³⁵. AIRs are prepared by homogenizing leaves and stems (from soil-grown plants) and roots (from liquid-cultured plants) in aqueous 80% EtOH followed by washes with absolute EtOH, chloroform-methanol, and acetone. Separate fractions containing RG-I, RG-II and oligogalacturonides can be obtained by size-exclusion chromatography (SEC) and ion exchange chromatography of the material solubilized from the cell walls by treatment with pectin methyl esterase (PME) and *endo*-polygalacturonase (EPG). The yields, glycosyl residue compositions, and glycosyl linkage compositions of each fraction can be determined²⁷.

The nucleotide and amino acid sequences of the fifteen *GALAT* gene family members are shown as follows.

Sequence #1 (SEQ ID NO:1)

5 Gene name: At3g61130

GeneBank accession # for reference: NM_115977 GI:18411855

Nucleotide sequence of Sequence #1:

Positions 1-2022 of CDS of NM_115977.

```

10      1 atggcgctaa agcgagggct atctggagtt aaccggatta gaggaagtgg tggtagatct
      61 cgatctgtgc ttgtcttct catattttc tgtgttttg cacctcttg cttcttgtt
      121 ggccgaggag tgtatatcga ttctcaa at gattattcaa ttgttctgt gaagcagaat
      181 ctgactgga gagaacgttt agcaatgcaa tctgttagat ctctttctc gaaagagata
      241 ctgatgtta tagcaaccag cacagctgat ttgggtcctc ttagccttga ttctttaag
15      301 aaaaacaatt tgtctgcatc atggcgggga accggagtag accctcctt tagacattct
      361 gagaatccag caactcctga tgtcaaatct aataacctga atgaaaaacg tgacagcatt
      421 tcaaaagata gtatccatca gaaagttgag acacctacaa agattcacag aaggcaacta
      481 agagagaaaa ggcgtgagat gcggggcaaat gagttagttc agcacaatga tgacacgatt
      541 ttgaaactcg aaaatgtctg cattgaacgc tctaagtctg ttgattctgc agtccttgg
20      601 aaatacagta ttggagaag agaaaatgag aatgacaact ctgattcaaa tatacgcttg
      661 atgcgggata aagtaataat ggctagagtc tatagtggga ttgcaaaatt gaaaaacaag
      721 aacgatttgt tacaagaact ccaggccga ctaaggaca gccaacgggt ttgggggaa
      781 gcaacatctg atgtgatct tctcggagt gcgcatgaga aactcagagc catgggtcaa
      841 gtcttgcta aagctaagat gcagttatat gactgcaagc tggttactgg aaagctgaga
25      901 gcaatgcttc agactgccga cgaacaagtg aggagcttaa agaagcagag tactttctg
      961 gctcagttag cagcaaaaac cattccaaat cctatccatt gcctatcaat gcgcttgact
      1021 atcgattact atcttctgtc tccggagaaa agaaaattcc ctggagtgaa aaacctagaa
      1081 aacctaatc ttatcatta tgccctctt tccgacaatg tattagctgc atcagtagtt
      1141 gtaactcaa ccatcatgaa tgccaaggat cttctaagc atgttttca cctgtcacg
30      1201 gataaactca atttcggagc aatgaacatg tggttcctcc taaaccacc cggaaaggca
      1261 accatacatg tggaacacgt cgatgagttt aagtggctca attcatctta ctgtcctgtc
      1321 ctctgtcagc ttgaatctgc agcaatgaga gactactatt taaagcaga ccatccaact
      1381 tcaggctctt cgaatctaaa atacagaaac ccaaagtatc tatccatgtt gaatcacttg
      1441 agattctacc tccctgaggt ttatccaag ctgaacaaaa tctcttctc ggacgatgac
35      1501 atcattgttc agaaagactt gactccactc tgggaagtta acctgaacgg caaagtcaac
      1561 ggtgcagtgc aaacctgtgg ggaaagtttc cacagattcg acaagtatct caactttcg
      1621 aatcctcaca ttgcgaggaa ctcaatcca aatgcttgtg gatgggctta tggaaatgaac
      1681 atgttcgacc taaaggaatg gaagaagaga gacatcactg gtatatacca caagtggcaa
      1741 aacatgaatg agaacaggac actatggaag ctagggacat tgccaccagg ataataaca
40      1801 ttctacggat taacacatcc cttaacaag gcgtggcatg tgctgggact tggatataac
      1861 ccgagtatcg acaagaagga cattgagaat gcagcagtgg ttactataa cggaacatg
      1921 aaaccatggt tggagttggc aatgtcaaaa tatcgccgtt attggacca gtaacatcaag
      1981 ttgatcacc catatcttcg tcgttgaac ctcatgaat aa

```

45

Amino Acid Sequence of Sequence #1: (SEQ ID NO:2)
 GeneBank ID# NP_191672
 Positions 1-673 of NP_191672.

5
 1 malkrglsgv nrirsggggs rsvlvlliff cvfaplcffv grgvvidssn dysivsvkqn
 61 ldwrerlamq svrsifskei ldviatstad lgplslsdfk knnlsaswrg tgvdpfrhs
 121 enpatpdvks nlnnekrdsi skdsihqkve tptkihrql rekrremran elvqhnddti
 181 lklenaaier sksvdsavlg kysiwrrene ndnsdsnirl mrdqvimarv ysgiaklnk
 10 241 ndllqelqar lkdsqrvlge atsdadlprs aheklramgq vlakakmqly dcklvtgklr
 301 amlqtadeqv rslkkqstfl aqlaaktipn pihclsmrlt idyylspek rkfrsenle
 361 npnlhyhalf sdnvlaasvv vnstimnakd pskhvfhlvt dklngamnm wflnppgka
 421 tihvenvdef kwlssycpv lrqlesaamr eyyfkadhpt sgssnlkymr pkylsmlnhl
 481 rfylpevypk lnkilfddd iivqkdltpl wevnlngkvn gavetcesf hrfdkylfns
 15 541 nphiarnfnp nacgwaygm n mfdlkewkkr ditgiyhwq nmnenrtlw lgtlppglit
 601 fygltphlnk awhvlglgyn psidkkdien aavvhyngnm kpwelelamsk yrpwytkyik
 661 fdhpylrrcn lhe

20 Sequence #2 (SEQ ID NO:3)

Gene name: At2g38650

GeneBank accession # for reference: NM_129422 GI:30687590

Nucleotide sequence of Sequence #2:

25 Positions 1-1860 of CDS of NM_129422

1 atgaaaggcg gaggcgggtg tggaggaggt ggtggcggag gaaaacgccg gtggaaagtt
 61 ctggtgattg gagtttgggt tctgttatt cttctatgc ttgttctct tgccttcta
 121 ctcggtcttc acaatggctt tcaactcct ggatttgtca ctgtcaacc ggcttctca
 30 181 ttgagagct ttaccagaat caatgctact aagcatacac agagagatgt atccgaacgg
 241 gtcgatgagg ttctcaaaa aatcaatcca gtcttccca agaaaagcga cataaacgtg
 301 ggtccagag atgtgaatgc aacaagcggc actgattcta aaaaaagagg attaccagtg
 361 tcccaactg ttgtgcaa tccaagccct gcaataaaa caaatcgga agcctcatat
 421 acaggtgttc agaggaaaat agtaagtgg gatgaaactt ggagaacttg tgaagtga
 35 481 tatgggagct actgcctctg gagggaggaa aataaggaac caatgaaaga tgccaaggtg
 541 aagcaaatga aggaccagct gttgtggct agagcactat atccagtat tgctaaaatg
 601 cttctcaaa gcaagtgac tcgggatag aaacagaata tccaagagtt tgagcgtatt
 661 cttagtgaag gtctcaaga tgctgacct ccaccacagg ttgataaaa gttgcagaag
 721 atggaagctg taattgcaaa ggcaaagtct ttccagtcg actgtaaca tgttgacaag
 40 781 aaattgagac agatccttga ttgactgag gatgaagcta gttccacat gaaacagagt
 841 gtgttctct accagcttgc agtacagaca atgcctaaga gtctcattg ctgtcaatg
 901 cgactaactg tggaacattt caagtcagat tcaattgagg atccattag tgagaaattt
 961 tcagatccct cattacttca cttgttatc atctccgata atatactagc atcgctccgt
 1021 gtgatcaact caacggttgt acatgcaagg gacagtaaaa acttgtttt ccatgtactg
 45 1081 acagacgagc agaattact tgcaatgaaa caatggttta ttaggaatcc tgcaaacaa
 1141 tcaactgttc aagtattgaa cattgaaaaa ctgagctgg acgattctga tatgaaactg
 1201 tcttgtctg cggagttccg tgttcttc cccagtggtg acctttggc gtctcaacg
 1261 aatagaacac actacttata ctttctct caatctcact atcttctcc caaattattt
 1321 gacaaattgg agaaggttgt gattctggat gatgacgttg tagtccagcg agacttatct
 50 1381 ccccttggg acctgatat ggaagggaaa gtgaatggcg ctgtaagtc gtgcactgtg

1441 agattgggtc agctaaggag tctcaagaga ggaaattttg ataccaatgc ttgtctctgg
 1501 atgtctggtt tgaatgtcgt tgatcttgc agatggaggg cattgggtgt ttcagaaacc
 1561 tatcaaaaat attataaaga gatgagtagt ggagatgagt cgagcgaagc aattgcattg
 1621 caggcaagct tgctcacatt tcaagaccaa gtatatgctc ttgacgacaa atgggctcta
 5 1681 tcagggcttg gttatgacta ctacatcaat gcacaagcca taaaaaacgc agccatattg
 1741 cactataacg ggaacatgaa gccgtggcct gagctgggaa tcccaaatta caaaaactat
 1801 tggagaaggc atctgagtcg ggaagatcgg ttctgagtg actgtaacgt gaatccttga

10 Amino Acid Sequence of Sequence #2: (SEQ ID NO:4)
 GeneBank ID# NP_565893
 Positions 1-619 of NP_565893.

1 mkgggggggg ggggkrrwkv lvigvlvli lsmivplafi lglhngfhsp gfvvqpass
 15 61 fesfrinat khtqrdvser vdevlqkinp vlpkksdinv gsravnatsg tdsckrglpv
 121 sptvvanpsp anktkseasy tgvqrkivsg detwrtcevk ygsyclwree nkepmkdakv
 181 kqmkdqlfva rayypsiakm psqskltrdm kqniqeferi lsessqdadi ppqvdkklqk
 241 meaviakaks fpvdcnnvdk klrqildite deasfhmkqs vflyqlavqt mpkslhclsm
 301 rltvehfksd sledpisekf sdpsllhfvi isdnilassv vinstvvhar dsknfvfhvl
 20 361 tdeqnyfamk qwfirnpckq stvqvlniek lelddsdmkl slsaefrvsf psgdllasqq
 421 nrthylsifs qshyllpklf dklekvvild ddvvvqrdls plwdldmegk vngavksctv
 481 rigqlrslkr gnfdtnaclw msglnvvdla rwralgvset yqkykemss gdesseaial
 541 qaslltfqdq vyaldkwal sglygydyin aqaiknaail hyngnmkpwl elgipnykny
 601 wrrhlsredr flsdcnvnp

Sequence #3 (SEQ ID NO:5)

Gene name: At5g47780

GeneBank accession # for reference: NM_124152 GI:30695292

30 Nucleotide sequence of Sequence #3:
 Positions 1-1851 of CDS of NM_124152.

1 atgatggtga agcttcgcaa tcttgttctt ttcttcatgc tctcaccgt cgttgcctat
 61 atccttctct acaccgatcc cgctgcctcc tcaagaccc ccttttctaa acgcgatttc
 35 121 ctcgaggacg taaccgcctt gactttcaat tccgatgaga atcgtttgaa tcttcttct
 181 cggaatctc ccgctgtgct cagaggagga ctgctcgggtg ctgtctattc cgataagaat
 241 tcacggcggc tagaccaatt gtctgctcga gttctttccg ccaccgacga tgatactcac
 301 tcacatactg acatttccat caaacaagtc actcatgatg cagcctcaga ctgcatatt
 361 aatagggaat atatgcatgt tcaattgacc caacaaacct ctgaaaaagt tgatgagcaa
 40 421 ccagagccta atgcttttgg agctaagaaa gatactggaa acgtgttgat gcctgatgct
 481 caagtgaggc atcttaaaga tcagcttatt agggcaaagg ttatctttc ccttccatct
 541 gcaaaggcca atgctcattt tggagagag ctgcgactcc gtattaaaga agtcaacgg
 601 gcacttgacg atgcctccaa ggattcggat ctgcaaaga ctgctataga aaagctaaaa
 661 gcaatggagc aaacactggc caaaggcaag cagatccaag atgactgttc tacagtggtc
 45 721 aagaagctac gtgctatgct ccactccgca gatgagcagc tacgggtcca taagaagcaa
 781 accatgtttt tgactcaatt gactgctaag accattccta aaggacttca ctgccttct
 841 ctgcgcctca ctacagacta ttatgcttta aattcatctg aacaacaatt tccaaatcag
 901 gagaaactag aagatactca gctgtatcac tatgcccttt tctctgataa tgttttggt
 961 acgtcagttg ttgttaactc taccataacc aatgcaaagc atcccttaaa gcatgtcttc
 50 1021 cacatcgta cagacagact caattatgcg gcaatgagga tgtggttcct ggacaatcca

1081 cctggcaaag ccaccatcca gggtcagaat gttgaagaat ttacatggct gaattcaagc
 1141 tacagtcccg ttctcaaaca gcttagttct agatcgatga tagattatta cttcagagcc
 1201 caccatacaa attcagacac caactgaag ttccggaatc caaaatactt atcgatcctt
 1261 aatcatcttc gttttactt gcttgagatc ttcccaagc tcagcaaagt gctctcttg
 5 1321 gatgatgata tagttgtgca gaaggacctt tctggctttt ggtcagttga tctgaaaggt
 1381 aatgttaacg gtgctgtaga gacgtgtggg gaaagcttc atcgcttga ccgttatctg
 1441 aacttctcaa atccactcat ttccaagaac ttgaccctc gagctgtgg ttgggcgtat
 1501 ggatgaatg tctttgatct ggatgaatgg aagaggcaaa acatcacaga agtttatcat
 1561 cgatggcagg atctgaatca agaccgagaa ttgtggaagc tagggacgtt gccgcctgg
 10 1621 ctaatcacat ttggagacg aacatatccg ctagaccgga aatggcacat actagggtt
 1681 ggatacaacc cgagtgtgaa ccaaagggat attgagaggg cagccgtgat acactataat
 1741 ggcaacctca aaccatggct agagattggg attccaagat acagaggctt ctggtaaag
 1801 catgtagact atgagcacgt ttatctcaga gaatgcaaca tcaatcctta g

15

Amino Acid Sequence of Sequence #3: (SEQ ID NO:6)

Genebank ID# NP_568688

Positions 1-616 of NP_568688.

20

1 mmvklrnivl ffmlltvah illytdpaas fktpfskrdf ledvtaltfn sdenrnlip
 61 respavirgg lvgavysdkn srldqlsar vlsatdddth shtdisikqv thdaasdshi
 121 nrenmhvqlt qqtsekvdeq pepnafgakk dtgnvlmpda qvrhlkdqli rakvylslps
 181 akanahfvre lrlrikevqr aladaskdsd lpktaieklk ameqtlakgk qiaddcstv
 25 241 kklramlhsa deqlrvhkkq tmfltqltak tipkglhclp lrlttdyyal nsseqqfnq
 301 ekledtqlyh yalfsdnvlv tsvvvnstit nakhplkhvf hivtdrlnya amrmwflndp
 361 pgkatiqvqn veefwlnss yspvlkqlss rsmidyyfra hhtnsdtnlk frnpkylsil
 421 nhrlfyppei fpklskvlfi dddivvqkdl sglwsvdlkg nvngavetcg esfhrfdryl
 481 nfnpliskn fdpracgway gmnvfdldew krqnitevyh rwqdlndre lwklgtlppg
 30 541 litfwrrtyp ldrkwhilgl gynpsvnqrd ieraavihyn gnlkpwl eig ipryrgfws
 601 hvdyehevlyr ecninp

Sequence #4 (SEQ ID NO:7)

35

Gene name: At1g06780

GeneBank accession # for reference: NM_100555 GI:30679825

Nucleotide sequence of Sequence #4:

Positions 1-1770 of CDS of NM_100555.

40

1 atgaaacaaa ttgctgatg gcagaggatt ttgatcctcg ctctgctatc gatatcagta
 61 ttgctccgc ttatttctg atcgaatcgg ctaagagca tcactcccgt tggctgtaga
 121 gaatttatg aagagttatc caaaattaga ttacagacaa atgacctag acttagcgct
 181 attgaacatg aggatggaga aggcttgaag gggccaaggc tcattctctt caaggatggg
 45 241 gagtttaatt cgtctgctga aagtgtggt ggtaatactt acaaaaacag ggaagaacaa
 301 gtgattgtt cacagaagat gacagttagc tctgatgaaa agggcctaat tctaccaaca
 361 gtcaaccaac ttgctaataa aacggatttc aagccccctt tatctaaggg tgaagaagaac
 421 acaagggttc agcccgacag agcaacagat gtgaaaacga aggagatcag agacaaaatt
 481 attcaagcta aagcctacct gaatttcgct ccacctggaa gtaactctca agttgtgaag
 50 541 gagttgagag gtcggctgaa agagctggaa cggctgtgtg gtgatgcaac aaaggacaag
 601 gacttatcaa agggcgctct ccgcagggtg aagcccatgg aaaatgtgtt atataaggct

661 agtcgtgtct ttaacaattg ccttgccatc gctaccaaac tccgtgccat gaattataac
 721 acagaagaac aagttcaggc gcagaaaaat caagcagcgt atctaataca gcttgcagca
 781 aggaccaccc caaaagggtc tctgtgtc tcaatgcggc tgacatcaga atactttca
 841 ctggatcctg aaaaaaggca gatgcctaac cagcaaaatt atttgacgc taatttcaat
 5 901 cattatgttg tcttctctga caatgtttg gcttcttcag tcgttgtaa ctctacgata
 961 tcttcatcaa aggagccaga aagaatagtc ttccatgtcg tgactgattc acttaattac
 1021 ccagcaatct caatgtggtt tctgctaaac attcaaagta aagctactat ccaaataccta
 1081 aacattgatg atatggatgt cctgcctaga gattatgac aattactgat gaagcaaaac
 1141 tctaataacc caagattcat ttctacactc aatcacgcac gcttctatct cccggatata
 10 1201 ttcccggtt tgaacaagat ggtactcttg gaccatgatg tagttgtca aagagattta
 1261 agtagactgt ggagcattga tatgaaagga aagggtggtg gagctgtaga gactgtctt
 1321 gaaggatgaat ctctatttcg atcaatgagc acatttata atttctcaga cacatgggtc
 1381 gctgggaaat ttatcctag agcttcgaca tgggcttcg ggatgaatct aattgatctc
 1441 gaagaatgga gaatacggaa gttgacttct acatacataa aatactcaa cctgggaaca
 15 1501 aagagaccat tgtggaaagc tgggagctta ccaatagggt ggtgacttt ctataggcaa
 1561 acattagcat tggacaagag atggcatgtg atggggtag gtcgcgaatc aggagtcaaa
 1621 gcggttgaca tcgaacaagc ggcagttata cactacgatg gggatcatgaa gccgtggtg
 1681 gacattgga aagagaatta caaacgttac tggaacatac acgtccctta ccatcacacc
 1741 tacttgcaac agtgcaatct tcaagcttga
 20

Amino Acid Sequence of Sequence #4: (SEQ ID NO: 8)

Genbank ID# NP_563771

Positions 1-589.

25 1 mkqirrwqri lilallsiv faplifvsnr lksitpvgrr efieelskir ftndlrlsa
 61 iehedgeglk gprlilfdg efnssaesdg gntyknreeq vivsqkmtvs sdegqilpt
 121 vnqlanktdf kplskgekn trvqpdratd vktkeirdki iqakaylnfa ppgsnsqvvk
 181 elrgrikele rsvgdatkdk dlsgalrrv kpmenvlyka srvfnncpai atklramnyn
 30 241 teeqvqaqkn qaaylmqlaa rtpkglhcl smritseyfs ldpekrqmpn qqnyfdanfn
 301 hyvvfsdnvl assvvvnsti ssskeperiv fhvvtlslny paismwfiln iqskatiqil
 361 niddmdvlpr dydqllmkqn sndprfistl nharfypdi fpglnkmlvl dhdvvvqrdl
 421 srlwsidmkg kvvgavetcl egessfrsms tfinfsdtwv agkfspract wafgmnlidl
 481 eewrirklt tyikyfnlgt krplwkagsl pigwltyrq tlaldrwhv mglgresgvk
 35 541 avdieqaavi hydgvmpwl digkenykry wnihvpyhht ylqqcnlqa

Sequence #5 (SEQ ID NO:9)

Gene name: At1g18580

GeneBank accession # for reference: AY062444 GI:17064735

5 Nucleotide sequence of Sequence #5:

Positions 1-1614 of CDS of AY062444.

```

1 atgaggcggg ggccgggtgga tcaccggcgg cgaggtagaa ggagattgtc gagttggata
10 61 tggttctcc ttggtcttt ctctgtcgt ggttagttc tctcatcgt tcagcattat
121 caccatcaac aagatccatc ccagcttta ctgagagag acacgagaac cgaaatggta
181 tctctcccc atttaaactt cacggaagag gtcacaagt cttctcctt ctctaggcag
241 ttgacagagc aatgacact tgccaaagct tatgtgtta tagctaaaga gcataataat
301 cttcatttag ctgggaatt gagttctaag atcagaagt gtcagcttt gctttccaaa
15 361 gcagctatga gaggacaacc tatttcgtt gatgaggcta aaccgattat tactggctta
421 tcagcttta tctacaaggc tcaagatgca cattaigata ttgccaccac tatgatgacc
481 atgaaatctc acatccaagc actgaagag cgtgcaaag cagctactgt tcagaccaca
541 atattgggc aatgggtgc tgaggcatta ccaagagcc tccactgtt gacgataaag
601 ctcacatctg attgggtaac agagccatct cgccatgaac tggcagatga gaacagaaac
20 661 tcacctagac ttgcgacaa caacctctac cacttctgca tcttctcgga caacgtgatt
721 gccacctcgg ttgtgttaa ttcaactgtc tcgaatgctg atcatccaaa gcagcttgtt
781 ttccacatag tgacgaatcg agtgagctac aaagctatgc aggctgggt tctaagtaat
841 gacttcaagg gtcagcaat agagtcagg agcgtagagg agtttcttg gttgaatgct
901 tcatattctc ctgtgttaa gcaactgctg gacacagatg caagagctta ctattcggg
25 961 gaacagacaa gtcaagatac gattccgag ccaaaagtga ggaacccaaa gtactgtca
1021 ttactgaacc atctcagatt ctacattccg gagatctatc cacagctaga gaagattgtt
1081 ttcttagacg atgatgtgtt tttcagaaa gatttgactc cactctctc ctgggatctg
1141 catggaaacg tcaatggagc tgtggaaaca tgtctgaag ctttcaccg atattacaag
1201 tatctaaatt tctcgaacc actcatcagc tcaaagtcg acccacaagc atgtggatgg
30 1261 gcttttgga tgaacgtttt tgatctgac gcttgaggga atgcaaacgt gactgctcgg
1321 taccattact ggcaagatca gaacagagaa cgaacgctt ggaaactcgg gacactccct
1381 ccaggctctac tatcttcta tggctcaca gagccactgg acagaagatg gcatgtctg
1441 ggttaggtt acgatgtgaa catcgataac cgtctgatcg aaacagcagc tgtgattcac
1501 tataatggta acatgaagcc ttggctaaag ctggctattg gtaggtataa accttctgg
35 1561 ttaaagttt tgaactcga ccatcctat ttacaagatt gtgtcacagc ttaa

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Amino Acid Sequence of Sequence #5: (SEQ ID NO: 10)

Genebank ID# AAK93644 GI:15293067

40 Positions 1-537 of AAK93644.

```

1 mrrwpvdhrr rrrrlsswi wflgsfsva glvlfivqhy hhqqdpsqll lerdtrtemv
45 61 spphlnftee vtsassfsrq laeqmtlaka yvfiakehnn lhlawelssk irscqllsk
121 aamrgqpisf deakpiitgl saliyaqda hydiattmmt mkshiqalee ranaatvqtt
181 ifgqlvaeal pkslhcltik ltsdwvtps rheladenrn sprlvdnnly hfcifsdnvi
241 atsvvvnstv snadhpqqlv fhivtrvsy kamqawflsn dfkgsaieir sveefswlna
301 syspvvkqll dtdarayyfg eqtsqdtise pkvrnpkyls llnhlrfyip eiypqlekiv
361 fldddvvvqk dltpifslid hgnvngavet cleafhryk ylnfsnplis skfdpqacgw
50 421 afgmnvfdli awrnanvtar yhywqdqnre rtlwklgtlp pgllsfygl epldrwhvl
481 glgydvnidn rlietaavii yngnmkpwik laigrykpfw lkflnssphy lqdcvta

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Sequence #6 (SEQ ID NO: 11)

Gene name: At2g20810

GeneBank accession # for reference: NM_127647 GI:30681142

5 Nucleotide sequence of Sequence #6:

Positions 1-1611 of CDS of NM_127647.

```

1 atgagaagga gaggagggga tagttccgg agagctggac ggaggaagat ctggaatgtg
61 gtatggtggg ttctctctgg tattgccctc ctgctcttct ttctcattct ctccaaagct
10 121 ggtcatattg aacctagacc ctctattcct aagcgacgtt accgtaatga caaatttga
181 gagggtatga atatgactga ggaaatgttg agtcctactt cgttgctcg tcaagttaat
241 gatcagattg ctcttgctaa agcttttgtt gtcattgcta aagaaagtaa gaatcttcag
301 ttgcttggg acitaaagtc tcagatccgt aactctcagt tgctttatc gagtgtgtgt
361 actaggagaa gtcccttgac tgccttgaa tctgagtcta ctattcgtga catggctgtt
15 421 ttgttatatc aagctcagca gcttcactat gatagtgtga ctatgattat gaggcttaag
481 gcctcgattc aggctcttga agaacaatg agttccgtta gcgagaagag ttccaagtat
541 ggacagattg ctgctgagga agtgcctaag agtctttact gtcttggtgt tcgtctcact
601 accgaatggg ttcagaattt agacttacag agaactctta aggaaaggag tcgtgttgat
661 tcgaaactca cggataacag tctctacat ttctgtgtgt ttccgataa cattattgct
20 721 acttctgttg tggtaattc tactgtcttc aattccaagg cccctgagaa agttgtgtt
781 catcttgtga ctaatgagat caactatgct gcaatgaagg ctgggtcgc cattaatatg
841 gacaacctca gaggagtac tgtggagggt cagaagttcg aggatttctc atggctgaat
901 gcttcctatg ttccggtcct caagcagctg caagactctg atacgcaaag ctattatttc
961 tctggacaca acgatgatgg gcgcactcca atcaaattca ggaaccccaa gtatctttcc
25 1021 atgtcaacc atcttaggtt ctacatccct gaagtgttc ctgcgtgaa gaaggtggct
1081 ttcttgatg atgatgtgt agttcagaag gatctttcat ctctctttc gatcgattta
1141 aacaaaaatg tgaacggggc tgttgagacc tgcatggaga cctccaccg ctaccacaag
1201 tacttgaact atttcatcc tctcatcgc tcccactttg atccagatgc gtgtgggtgg
1261 gcgtttggaa tgaacgtctt tgatttagtt gagtggagga agagaaatgt gaccggcata
30 1321 taccactact ggcaagaaaa aaacgtggac cggaccttat ggaaactggg aacactacct
1381 ccaggacttc tgacatttta cgggttaaca gaggcactag aggcgtcctg gcatatcctg
1441 ggattgggat acacgaatgt gtagtctcgt gtgatagaga aaggagctgt tcttcacttc
1501 aatgggaact taaagccatg gttgaagatc gggatagaga agtacaacc tttgtgggag
1561 agatacggtg attacacttc tcctttatg caacaatgca atttcattg a

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35 Amino Acid Sequence of Sequence #6: (SEQ ID NO: 12)

Genebank ID# NP_565485

Positions 1-536 of NP_565485.

```

40 1 mrrrggdsfr ragrrkisnv vwwvlsgial llffliska ghieprpsip krryrndkfv
61 egmnmteeml sptsvarqvn dqialakafv viakesknlq fawdlsaqr nsqllssaa
121 trrspitvle sestirdmav llyqaqqlyh dsatmimrlk asiqaleeqm ssvsekssky
181 gqiaaeevpk slyclgvrlt tewfnldlq rtlkrsrd skltdnslyh fcvfsdnia
241 tsvvvnstal nskapekvfv hlvneinya amkawfainm dnlgvtvfv qkfedfswln
45 301 asyvpvlkql qdsdtqsyf sghnddgrtp ikfrnpkyls mlnhlrfyip evfpalkkv
361 fldddvvvqk dlsslfsidl nknvngavet cmetfhryhk ylnyshplr shfdpdacgw
421 afgmnvfdlv ewrkrnvtgi yhywqeknvd rtlwklgtlp pglftfygt ealeaswhil
481 glgytnvdar viekgavlhf ngnlkpwiki giekykplwe ryvdytspfm qqcnfh

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Sequence #7 (SEQ ID NO: 13)

Gene name: At2g30575

GeneBank accession # for reference: NM_179819 GI:30684641

5 Nucleotide sequence of Sequence #7:

Positions 1-1833 of NM_179819.

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1 atgaatcaag ttcgtcgttg gcagaggatt ctgatcctct cgctgctatt gttatctgtt
61 tttagctccga ttgttttcgt ttgaatcgg ctaagagca tcaactccgt cgatagagga
10 121 gaattcattg aagaattatc cgacattaca gataagaccg aggatgaact tagacttact
181 gctattgaac aggacgaaga aggctgaag gagcctaaac gtattctgca ggcagagat
241 ttaattctg tggtttgc aaattcctct gataaaagta atgatactgt gcagtcta
301 gagggagacc aaaaaaactt tcttcagaa gttgataagg gaaataatca caaaccaaag
361 gaggaacaag cagtttcaca gaaaaccaca gtaagctcga atgcggaggt gaaaatttca
15 421 gcaagagata ttcaacttaa tcataaaacg gaattccgac ccccttcaag taagagtga
481 aagaatacaa gggttcaact tgaaagagca acagatgaga gggtaaagga gatcagagac
541 aaaattatcc aagcgaaagc ctatctgaat ttggccctac ctgggaataa ctcccaaact
601 gtaaaggagt tgagagttcg aacgaaagag ctggaacggg ctactggtga tactaccaag
661 gataaatatt tgcaaagag ctctcctaac agattgaagg ccatggaagt tgcgttatac
20 721 aaggtcagcc gtgcctttca caactgccct gccattgcta ccaaactcca agccatgact
781 tataaaaccg aagaacaagc tcgggcgcag aagaacaag cagcatattt aatgcagctt
841 gcagcaagga ctaccccaaa agggcttcat tgtctctcaa tgcggttgac aacagaatat
901 ttaccctgg atcacgaaaa aaggcagctt ttgcaacaaa gttataatga tctgatctc
961 taccattacg tagtcttctc tgacaatgtt ttggcctctt cgggtgtgtg taactctaca
25 1021 atctctcat caaaggaacc ggataaaata gtaattccatg tggtgacaga ttactcaat
1081 taccagcaa tctcaatgtg gttttacta aaccaagtg gcagagcttc aatccaaatc
1141 ctaaacattg atgaaatgaa tgcctgccca ttgtaccatg ctgaattgct gatgaagcaa
1201 aattcaagtg acccaagaat catttcagcg ctcaaccatg cacgcttcta tctccagat
1261 atcttcccag gtctaaacaa gatcgctact ttcgatcatg atgtagtagt gcaaagggat
30 1321 ctaactagac tgtggagcct tgatatgacg gggaaagtgt ttggagctgt agagacttgt
1381 ctgaagggtg atccttcata tcgttcgatg gactcattca ttaatttctc agatgcatgg
1441 gtttctcaga aatttgatcc caaggcttgc acttgggcat tcgggatgaa tctatttgat
1501 ctgaagaat ggagaagaca ggagttgact tctgtatacc tgaaatactt cgacctggga
1561 gtaaaaggac atctgtggaa agcaggggga ttgccagtag gttggttgac tttttcggg
35 1621 caaacgttgc cgttggaata gagatggaac gtgggtgggt taggtcacga atcaggactc
1681 agggcaagcg acatcgaaca agcagcggtt atacactacg acgggatcat gaaaccatgg
1741 ctggacatcg gtatagacaa gtacaagcgc tactggaaca tacatgtacc ttaccatcac
1801 ctcacttac aacggtgcaa cattcacgat tga

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40

Amino Acid Sequence of Sequence #7: (SEQ ID NO: 14)

Genebank ID# NP_850150

Positions 1-610 of NP_850150.

45

50

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1 mnqvrwqri lilslllsv lapivfsnr lksitsvdrq efieelsdit dktedelrlt
61 aieqdeeglk epkrlqdrd fnsvvlss dksndtvqsn egdqknflse vdkgnnhkpk
121 eeqavsqktt vssnaevkis ardiqlnhkt efrppsskse kntrvqlera tdervkeird
181 kiiqakayln lalpgnnsqi vkelrvrke leratgdtk dkylpksspn rlkamevaly
241 kvsrafhncp aiatklqamt ykteeqaraq kkqaaylmql aarttpkglh clsmrltley
50 301 ftdhekrql lqsyndpdl yhyvvfsdnv lassvvvnst issskepdki vfhvvtdsln

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361 ypaismwfl npsgrasiqi lndemnvlp lyhaellmkq nssdpriisa lnharfylpd
 421 ifpglnkivl fdhdvvvqrd ltrlwsldmt gkvvgavetc legdpsysrm dsfinfsdaw
 481 vsqkfdpkac twafgmnlfd leewrrqelt svylkyfdlg vkghlwkagg lpvgwlffg
 541 qtfplekrwn vgglgghesgl rasdieqaav ihydgmikpw ldigidkykr ywnihvpyhh
 5 601 phlqrcnihd

Sequence #8 (SEQ ID NO: 15)

Gene name: At2g46480

10 GeneBank accession # for reference: NM_130212 GI:22326493

Nucleotide sequence of Sequence #8:

Positions 1-1587 of NM_130212.

1 atgactgatg cttgttgtt gaagggaaac gaggacaaaa tggttcctcg ttttggtcat
 15 61 ggaacctgga taggaaaagc attaatgat acaccagaga tgttgcata aaggagtctg
 121 agacaggaaa aaagattgga aagggctaag gagctgatga atgatgatag tctgcaaaaag
 181 ctgagacgg cagcatggc acgttccaga tctgtcgatt ctgcaccact aggaaactac
 241 accatttga aaaatgaata ccggaggggc aagagtttg aagatatgtt acgtttgatg
 301 caagatcaaa tcatcatggc acgagtttac agtggacttg caaagtttac aaacaatctc
 20 361 gccttgcacc aagagataga aacacaacta atgaaactag cttgggagga agaattact
 421 gatattgatc aggagcagag agtacttgac agtataagag acatgggaca aatactggct
 481 agagcacacg agcagctata tgaatgcaag ttggtgacaa ataagttgag agcaatgcta
 541 caaacagttg aagatgaact cgaaaacgag cagactata taacgttctt gactcagcta
 601 gctccaagg cactaccaga tgctatccac tgctgacca tgcgctgaa tctagagtat
 25 661 catctcctgc cttaccgat gagaaatgtt ccaaggaggg agaatttga gaatccaaaa
 721 cttaccact acgtctctt ctctgataat gtactggctg catcagttgt tgtcaactcc
 781 acagtcatga atgcacagga tcttcaagg catgtttcc acctgtgac tgataagctc
 841 aactttggag caatgagtat gtggtttctg ttgaaccctc ctggagaagc gaccatccat
 901 gtccaaaggt ttgaagattt tacttggctc aactcatctt actctccagt ttgagtcag
 30 961 ctgagtcag cagctatgaa gaagtctac tcaagacag cgaggtctga atcagttgaa
 1021 tcaggctcag aaaacctcaa gtaccggtac ccgaaataca tgtcaatgct taaccacctg
 1081 aggttctaca tccctaggat ctcccaaag ttggagaaaa tctgtttgt tgacgatgat
 1141 gtggttgtc agaaggattt aactccccta tggccattg atcttaaagg gaaagtgaat
 1201 gaaaactttg atcccaagtt ctgcggatgg gcttatggga tgaacatctt cgacctgaaa
 35 1261 gaatggaaga agaacaacat tacagaaact tatcactttt ggcaaaacct gaacgaaaac
 1321 cggactctat ggaaactagg aacattgcca ccagggtcga taacgttcta caatctgaca
 1381 caaccacttc agagaaaatg gcactactt ggactgggtt atgataaagg aatcgatgtc
 1441 aagaagattg aaagatcagc tgttatcat tacaatggac acatgaaacc atggacagag
 1501 atggggataa gcaagtatca gccatattgg acgaagtaca ccaatttga ccatccttac
 40 1561 atctttactt gcaggctgtt tgagtga

Amino Acid Sequence of Sequence #8: (SEQ ID NO: 16)

Genebank ID# NP_182171

45 Positions 1-528 of NP_182171.

1 mtdacclkg edkmvprfgh gtwigkafnd tpemlhersl rqrklerin elmnddsllqk
 61 letaamars svdsaplgn tyiwkneyrrg ksfedmlrlm qdqiimarvy sglakftnnl
 121 alhqietql mklaweeest didqevrld sirdmgqila raheqlyeck lvtnkrlaml
 50 181 qtvedelene qtyitftql askalpdaih cltmrlnley hllplpmrnf prrenlenpk

241 lyhyalfsdn vlaasvvvns tvnnaqdpsr hvfhlvtdkl nfgamsmwfl inppgeatih
 301 vqrfedftwl nssyspvlsq lesaamkkfy fktarsesve sgsenlkryr pkymsmnlhl
 361 rfyprifpk lekilfvddd vvvqkdltp lwsidlkgvn enfdpkfcgw aygmnlfdlk
 421 ewkknitet yhfwqnlne rllwklgtlp pglitfynlt qplqrkwhll glgydkgidv
 5 481 kkiersavii ynghmkpwte mgiskyqpyw tkytnfdhpy iftcrife

Sequence #9 (SEQ ID NO: 17)

Gene name: At3g01040

10 GeneBank accession # for reference: NM_110969 GI:30678269

Nucleotide sequence of Sequence #9:

Positions 1-1602 of CDS of NM_110969.

15 1 atgcagcttc acatatacgcc tagcatgaga agcattacga tatcgagcag caatgagttt
 61 attgatttga tgaagatcaa agtcgcagct cgtcacatct ctaccgaac tctctccac
 121 actatcttaa tctcgccttt ctgtttacct ttgttttca tctaaccgc tgtgttacc
 181 ctgaaggtg tcaacaagtg ctctctttt gattgtttcg ggaggcggct aggaccacgt
 241 ctcttggtg gtagataga ttacagagcag agactagtta gagattttta caaatttcta
 20 301 aatgaagtaa gactcaaga aattccagat ggtttaaagc ttccagagtc tttagtcaa
 361 ctggttcgg atatgaagaa caaccactat gatgctaaaa cattgacct cgtatttga
 421 gctatgtag agaagttga aagggttta aggaatcca aattgcaga actcatgaac
 481 aagcactttg ctgcaagttc aattcaaaa ggaattcact gtctctctt aagactaacc
 541 gatgaatatt cctcaatgc tcatgcccgg agacagcttc ctccccgga gctctccct
 25 601 gttctctcag acaatgctta ccaccatttt gttctagcta cagataatat cttagctga
 661 tgggtgttg tctcatctgc tgttcaatca tctcaaaac ccgagaaaat tgtcttccat
 721 gttatcacag acaagaaaac ctatgcgggt atgcattctt ggttgcact caattctgt
 781 gctctgcga ttgtgaagt gaaaagcgt catcagttt attggttaac aagagagaat
 841 gttccagttc tgaagctgt ggaaagccat aacagtatca gaaattatta ccatgggaat
 30 901 catattgctg gtgcaaacct cagcgaaca acccctcga catttgctc gaaactgcag
 961 tcaagaagtc ccaatacat atcttgcct aacctctta gaatatatc accagagctt
 1021 ttccgaact tagacaaggt agtgttctta gatgatgata tagtgataca gaaagattta
 1081 tctccgcttt gggatattga cctaacggg aagggttaag gagctgtgga gactgtcga
 1141 ggagaagacg tatgggttat gtcaaagcgt cttaggaact actcaattt ttctacccg
 35 1201 ctcatcgcaa agcatttaga tcccgaagaa tgtgcttggg ctatggaat gaatatctt
 1261 gatctacgga ctggaggaa gacaaatc agagaaacgt atcattctg gctaaagag
 1321 aatctgaagt cgaatctaac aatgtgaaa ctggaacat tgctcctgc tcaatagca
 1381 tttaaaggtc atgttcagcc aatagattcc tctggcata tgcctggatt aggttatcag
 1441 agcaagacca acttagaaaa tgcgaagaaa gctgcagtga ttattacaa tggccaatca
 40 1501 aagccgtggc ttgagatagg ttctgagcat ctgagacat tctggacaaa atatgttaac
 1561 tactccaatg atttcattaa gaattgcat atcttgaat ag

Amino Acid Sequence of Sequence #9: (SEQ ID NO: 18)

45 Genebank ID# NP_186753

Positions 1-533 of NP_186753.

1 mqlhispsmr sitisssnef idlmkikvaa rhisyrtlfh tililafllp fviltavvt
 61 legvncsssf dcgrrlgr llgriddseq rlvrdfykil nevstqepd glklpesfsq
 50 121 lvsdmknnhy daktfalvfr amvekferdl reskfaelmn khfaassipk gihclslrt
 181 deysnabar rqlpspellp vlstdnayhhf vlatdnlaa svvvssavqs sskpekivfh

241 vitdkktyag mhswfalnsv apaivevksv hqfdwltren vpvleavesh nsimyyhgn
 301 hiaganlset tprtfasklq srspsyisll nhliylpel fpnldkvvfl dddiviqkdl
 361 splwdidlng kvngavetcr gedvwvmskr lrnyfnfshp liakhldpee cawaygmni
 421 dlrtwrktni retyhswlke nlksnltnmwk lgtlppalia fkghvqpids swhmllggyq
 5 481 sktnlenakk aavihyngqs kpwlfigfeh lrpfwtkyvn ysndfiknch ile

Sequence #10 (SEQ ID NO: 19)

Gene name: At3g02350

10 GeneBank accession # for reference: NM_111102 GI:18396158

Nucleotide sequence of Sequence #10:

Positions 1-1686 of CDS of NM_111102.

1 atggcgggtgg ccttccgtgg aggccgggga ggcgtcggat cggccaatc taccggactt
 15 61 cgtagtttct tctcctaccg gatctttatc tccgcttgt tctctttct cttcctcgcc
 121 acttttccg tcgttctaa ctctctctgt catcagcctc atcaggatca tacattgccg
 181 agtatgggca acgcatatat gcagaggacg ttttggctt tgcaatcgga tccattgaaa
 241 actaggttgg atctgataca caagcaagcc attgatcatt tgacactggg gaatgcgat
 301 gctgcttacg ctaggaagct aaagcttgat gcttctaagc agcttaagct cttcgaagat
 20 361 ttggctatca acttctcgga ttgcagtcg aaacctggtt tgaaatctgc tgtgtctgat
 421 aatggtaatg ctcttgagga ggattcgtt aggcagcttg agaaagaagt gaaggataag
 481 gtgaagacag cgaggatgat gatcgttgag tctaaagaga gttatgatac acagcttaaa
 541 atccagaagt tgaaagatac aatctttgct gtccaagaac agtgacaaa ggctaagaaa
 601 aacggtgagg ttgctagctt gatttcagcc aagtcgggtc ctaaaagtct tcattgtttg
 25 661 gccatgagcg ttgtaggaga gaggatctct aatcctgaga agtacaagga tgctccacct
 721 gaccagccg cagaggatcc aactctttac cactatgcga ttttctctga taatgtcatt
 781 gctgtgtctg ttgtggtgag atcgggttg atgaacgctg aggagccatg gaagcatgct
 841 ttccatgtgg tgacagatcg gatgaatctc gcagccatga aggtgtggtt taagatgcgt
 901 ccttggacc gtggtgcca tgttgagatt aaatccgtgg aggattcaa gttcttaaac
 30 961 tcttctatg cgccggtctt gaggcagctt gagtctgcca agtgcagaa gtttacttt
 1021 gagaatcaag ctgagaacgc aactaaagat tcacataacc tcaagttcaa gaaccccaag
 1081 tatctctcga tgtgaacca tctcagattt tacttaccag agatgtatcc gaagctgaat
 1141 aagattttgt tcttgacga tgatgttggt gtgcagaaag acgtgactgg tttatggaaa
 1201 atcaacttgg atggcaaggt gaatggagcc gttgagacat gtttgggtc tttcatcga
 35 1261 tatggtcaat acttaaacct ctctcatcct ttgatcaaag agaactttaa cccagtgcc
 1321 tggcttggg ctttggaaat gaacataatc gatcfaatg cctggagacg cgagaagtgc
 1381 accgatcaat accattactg gcagaacctg aatgaagaca gaactctctg gaaattggga
 1441 actctacctc cgggattgat cacattctat tcaaagacga aatcattgga caaatcatgg
 1501 catgtacttg gtttaggcta taacccggga gtgagcatgg acgaaatcag aaatgcagga
 40 1561 gtgattcatt acaatggaaa catgaaaccg tggctagaca ttgcgatgaa ccaatacaag
 1621 tctctctgga ctaaatatgt tgataacgaa atggagtttg tgcagatgtg caattttggt
 1681 ctctaa

45

Amino Acid Sequence of Sequence #10: (SEQ ID NO: 20)
 Genebank ID# NP_566170.1
 Positions 1-561 of NP_566170.

5 1 mavafrggrg gvgsgqstgl rsfssyrifi salfsflfla tfsvlnssr hqphqdhtlp
 61 smgnaymqrt flalqsdplk trldlihkqa idhltlvnay aayarklkld askqlklfed
 121 lainfsdlqs kpglksavsd ngnaleedsf rlekevkdk vktarmmive skesydtqlk
 181 iqlklkdifa vqeqltkakk ngavasalisa ksvpkslhcl amrlvgeris npekykdapp
 241 dpaaedptly hyaifsdnvi avsvvrvsvv mnaeepwkhv fhvvtdrnml aamkvwfkmr
 10 301 pldrghavei ksvedfkfln ssyapvlrql esaklqkfyf enqaenatk dshnlkfknpk
 361 ylsmlnhlrf ylpemypkln kilfldddvv vqkdvgtlwk inldgkvnga vetcfgsfhr
 421 ygqylnfshp likenfnpsa cawafgmni dlnawrrekc tdqyhywqnl nedrtlwklg
 481 tlppglitfy sktksldksw hvlglgynpg vsmdeirnag vihynngmkp wldiamnqyk
 541 slwtkyvdne mefvqmcnfg l

15 Sequence #11 (SEQ ID NO: 21)

Gene name: at3g25140

GeneBank accession # for reference: NM_113418 GI:30687767

20 Nucleotide sequence of Sequence #11:
 Positions 1-1680 of CDS of NM_113418.

 1 atggctaatac accaccgact ttacgcggc ggcggatctc cggccataat cgggtggcaga
 61 atcacactca cagctttcgc ttccactatc gcactcttc tctcactct ctctctctc
 25 121 ttgcgttcag attctaacga ttctctgat ctctctctc ccggtgtga gtactcta
 181 ggagtcggat ctagaagatc catgttggat atcaaacgg atccgctta gccacgggtg
 241 attcagatcc ggaaacaagc tgatgatcat cggtcattag cattagctta tgcttctac
 301 gcgagaaagc ttaagctcga gaattcgaaa ctgctcagga tcttcgctga tcttcgagg
 361 aattacacgg atctgattaa caaacggacg tatcgagctt tgatgattc tgatggagcc
 30 421 tgcattgaag aatctgtgct taggcaattt gagaaagaag ttaaggaacg gattaaaatg
 481 actcgtcaag tgattgctga agctaaagag tctttgata atcagtgaa gattcagaag
 541 ctgaaagata cgattttcgc tgtaacgaa cagtaacta atgctaagaa gcaagggtgc
 601 ttttcgagtt tgatcgctgc gaaatcgatt ccgaaaggat tgcattgtct tgctatgagg
 661 ctgatggaag agaggattgc tcaccctgag aagtatactg atgaaggga agatagaccg
 35 721 cgggagctcg aggatccgaa tctttaccat tacgtatat ttcggataa tgtgattgcg
 781 gcttcgggtg ttgtgaactc tgctgtgaag aatgctaagg agccgtggaa gcatgtttt
 841 cactgtgtga ctgataagat gaatcttga gctatgcagg ttatgttaa actgaaggag
 901 tataaaggag ctcatgtaga agttaaagct gttgaggatt atacgtttt gaactctcg
 961 tatgtgcctg tgtgaagca gttagaatct gcgaatctc agaagttta ttcgagaa
 40 1021 aagctcgaga atcgacgaa agataccacg aatatgaagt tcaggaaccc caagtattta
 1081 tctatattga atcacttgag gttttattta cccgagatgt acccgaaact acataggata
 1141 ctgttttgg acgatgatgt ggtgtgcag aaggatttaa cgggtctgtg ggagattgat
 1201 atggatggga aagtgaatgg agctgtagag actgttttg ggtcgttca tcggtacgct
 1261 caatacatga atttctaca tctttgatc aaagagaagt ttaatccaa agcatgtgcg
 45 1321 tgggcgtatg gaatgaactt ctttgatctt gatgcttga gaagagagaa gtgcacagaa
 1381 gaatatcact actggcaaaa tctgaacgag aacagggctc tatggaaact ggggacgtta
 1441 ccaccgggac tgatcacctt ttactcaacc acaaagccgc tggacaaatc atggcatgtg
 1501 cttgggctgg gttacaatcc gagcattagc atggatgaga tccgcaacgc tgcagtggta
 1561 cacttcaacg gtaacatgaa gccatggctt gacatagcta tgaaccagtt tcgaccactt
 50 1621 tggaccaaac acgtcgacta tgacctcgag ttgttcagg cttgcaattt tggcctctga

Amino Acid Sequence of Sequence #11: (SEQ ID NO: 22)

Genebank ID# NP_189150

Positions 1-559 of NP_189150.

5 1 manhhrlrg ggspaiigr itltafasti alfltlff fasdsndspd llpgveysn
 61 gvgsrrsml d ikdplkprl iqirkqaddh rslalayasy arklklensk lvrifadlsr
 121 nytdlinkpt yralydsdga sieesvlrqf ekevkerikm trqviaeake sfdnqlkiqk
 181 lkdtifavne qltnakkqga fssliaaksi pkglhclamr lmeeriahp kytdegkdrp
 241 reledpnlyh yaifsdnvia asvvvnsvak nakepwkhvf hvvtdkmnlg amqvmfklke
 10 301 ykgahvevka vedytlfnss yvpvlkqles anlqkfyfen klenatkdt nmkfrnpkyl
 361 silnhlrfyl pemypklhri lfdddvvvq kdlglweid mdgkvngave tcfgsfhrya
 421 qymnfshpli kekfnpkaca waygmnfddl dawrrekcte eyhywqnlne nralwklgtl
 481 ppglitfyst tkpldkswhv lglgynpsis mdeirnaavv hfngnmkpwl diamnqfrpl
 541 wtkhvdyle fvqacnfgl

15

Sequence #12 (SEQ ID NO: 23)

Gene name: At3g58790

GeneBank accession # for reference: NM_115741 GI:22331856

20 Nucleotide sequence of Sequence #12:

Positions 1-1623 of CDS of NM_115741.

 1 atgaagtttt acatatcagc gacggggatt aagaaggtta cgatatcaaa tcccggcgtc
 61 ggaatcggta aaggaagcgg aggatgtgcg gctgcagcgg cggcgttagc agcgcgagaga
 25 121 ttctctagtc gcacgttgtt actgttgctg ctgctgctcg ctatcgtcct ccctttatc
 181 ttgcgcaggt tcgcgtttct cgtcctcgaa tctgcctccg ttgcgattc accactcgat
 241 tgcattgggac tcagactttt ccgtgggggc gacacatctc tgaaaattgg ggaagagttg
 301 acacgggctc tagtgaaga gacgacagat catcaggacg ttaatggaag aggaacgaag
 361 ggatcattgg agtcattcga cgacctgtt aaggagatga cgtaaaacg ccgtgacata
 30 421 agggcggttg cttccgtgac taagaagatg ctgtgcaga tggaacgtaa agtccaatca
 481 gcgaacatc atgagttagt gtactggcat ttagcctctc acggtattcc taaaagcctc
 541 cattgccttt ccctcagatt aactgaagag tactctgtaa atgcaatggc tcgaatgcgt
 601 ttgcctccgc ctgagtcctg atcacgtctg accgacccat ctttcatca tattgtcctc
 661 ctgactgaca atgtccttgc tgcctctgtc gtcatatcgt ctactgtaca aaacgctgtg
 35 721 aatccccgaga agtttgtctt tcatattgtt accgataaga aaacctatac ccctatgcat
 781 gcttggtttg ctatcaactc tgcttcatca ccagttgttg aagtaaaggg acttcatcag
 841 tatgatggc ctcaagaagt gaactcaaa gtagagaga tgctggacat tcaccgctta
 901 atttgagac gacattatca aaattgaaa gactctgatt ttagtttgt tgagggtact
 961 catgagcagt ccttgcaagc tctaaatcct agctgccttg ccctttgaa ccatcttgc
 40 1021 attacattc ccaagctttt tccagatctc aacaagatag tgtgttgga tgatgatga
 1081 gtagtacaga gcgatcttc gtctttatgg gaaacggatc tcaacggtaa agttgttgt
 1141 gctgtcgttg attcgtggtg cggagacaac tgttgccccg gaagaaaata caaagactat
 1201 ttcaacttct cacatcctt gatctcatca aacttagttc aagaagactg tgcttggtt
 1261 tctggatga atgtcttga tctcaaagcc tggagacaaa ccaatattac tgaagcttac
 45 1321 tctacatggc taagactcag tgtaggtca ggactacaat tatggcaacc aggggcttta
 1381 ccaccgacat tacttgctt caaaggactt acacagtctc tgaaccatc atggcacgtc
 1441 gctggactag gttctcgatc cgtaaaatcc cctcaagaga ttctgaaatc tgcttcggtt
 1501 ttacatttca gcggtccagc aaaaccgtgg ctagagatca gtaaccctga ggtacgatct
 1561 ctttggtata gatacgtaaa ttctccgac atcttcgtta gaaaatgcaa aatcatgaac
 50 1621 tga

Amino Acid Sequence of Sequence #12: (SEQ ID NO: 24)

Genebank ID# NP_191438.2

Positions 1-540 of NP_191438.

5 1 mkfyisatgi kkvtisnpgv gigkgsggca aaaaalaarr fssrtlllll llaivlpfi
 61 fvrfaflvle sasvcdspld cmglrlfrgg dtslkigeel tralveetd hqdvngrgtk
 121 gslesfddlv kemtlkrrdi rafasvttkm llqmerkvsq akhhelvywh lashgipksl
 181 hclslrltee ysvnamarmr lpppesvsrl tdpshhivl ltdnvlaasv visstvqnav
 241 npekfvlhiv tdkktytpmh awfainsass pvvevkgllh ydwpqevnfk vremldihrl
 10 301 iwrhyqnkl dsdfsvegt heqslqalnp sclallnhlr iyipklfpdl nkivldddvd
 361 vvqsdlsllw etdlnkvvg avdswcgn cccprkykdy fnfshpliss nlvqedcawl
 421 sgmnvfdlka wrqtniteay stwrlsvrs glqlwqpgal pptllafkgl tqslepswhv
 481 aglgsrsvks pqeilksasv lhfsgpakpw leisnpevrs lwryvnnssd ifvrckkimn

15 Sequence #13 (SEQ ID NO: 25)

Gene name: At4g38270

GeneBank accession # for reference: NM_119989 GI: 30691874

Nucleotide sequence of Sequence #13

20 Positions 1-2043 of CDS of NM_119989.

 1 atgacgacgt tctctacatg cgccgccttt ttatcgctgg tagtagtgct acatgctgtt
 61 catgtcgggt gagccatttt agagtcacaa gcacccca gagaacttaa agcttatcgt
 121 ccgctgcaag ataataatct acaggagggt tatgtctcct cagctgctgc agtgactac
 25 181 gatccagatc tgaaagatgt gaacatagtt gcgacataca gtgaccatta cggcaatata
 241 cgccttggtg gggtgaaaat gggggatcct tcaccttctt gggttttgga gaatcctgcc
 301 tatcaagtta gccgcaaaac aaaagggtcg cagctagtta taccacggga ttcatitcaa
 361 aatgatactg gaatggaaga taatgcaagc cattctacaa ctaatcagac tgatgaaagc
 421 gaaaatcagt ttccaaacgt ggattttgca agcccagcaa aactgaagcg gcagatttta
 30 481 cgtcaggaaa ggagagggtc acgaacttta gagctgatcc gacaagaaaa ggaaactgat
 541 gagcagatgc aagaagcagc cattcagaag tcaatgagct ttgaaaactc agtcataggg
 601 aaatacagta tatggaggag agactatgag agcccaaag ctgatgctat ctgaagctt
 661 atgagagacc agatcataat ggcaaaagca tatgcaaata ttgcaaatac aaaaaatgta
 721 accaatctgt acgttttctt gatgcagcag tgtggagaaa ataaacgtgt tataggtaaa
 35 781 gcaacctctg atgtgacct tccttcaagc gctctgatc aagcaaaagc catgggcat
 841 gcactctctc ttgcaaaaga cgagtatat gactgccatg aacttgcaaa aaagtccgg
 901 gccatccttc agtcactga acgcaaagta gatggactga agaaaaaggg aaccttcta
 961 attcagctag ctgcaaaaac atttccaag ccattgcatt gcctgagtct gcagctagcg
 1021 gcagactatt ttattctagg ttcaatgaa gaggatgcag tgaaagagga tgcagtaaa
 40 1081 aagaagcttg aagatccttc gctctatcac tatgcgatct ttccggataa cggtctggct
 1141 acatcagtggt tggggaactc cactgtcttg aatgcaaagg aaccgcagag gcattgttct
 1201 catatagtaa ctgacaaact gaattttggt gcaatgaaga tgggtttcg catcaatgct
 1261 cctgctgatg cgacgattca agttgaaaac ataatgatt tcaagtggct gaactcctct
 1321 tactgtctgt ttctacggca gcttgaatct gcaaggctga aagaatacta ttcaaagca
 45 1381 aatcatcctt catcaatctc agctggcgca gataatctaa agtaccgcaa ccaaagtat
 1441 ctatcgatgc tgaatcatct cagattctac ctctcgagg ttatccgaa gctggagaag
 1501 attctgttct tagacgatga catttggttg cagaaggacc tggcaccact atgggaaata
 1561 gacatgcaag gaaaagttaa tgggtcggtg gagacgtgca aggagagctt ccacagattt
 1621 gacaagtacc tcaactctc aaatccaaag atttcagaga atttgacgc tgggtgctgt
 50 1681 ggggtggcat ttgggatgaa tatgtttgac ctgaaagagt ggaggaaacg gaacattaca

1741 gggatatatc actattggca agacttgaat gaagacagaa cactgtggaa gctgggatcg
 1801 ttgccaccgg ggctgataac atttacaac ctgacgtatg caatggatag gagctggcac
 1861 gtactagggc tgggatatga cccagcgcta aaccaaacag caatagagaa tgcagcggta
 1921 gtgcattaca atgggaacta caagccatgg ctgggttag cattcgcaa gtacaaaccg
 5 1981 tactgggtcca agtacgttga gtacgacaac ccttatctcc gacggtgcga catcaatgaa
 2041 tga

Amino Acid Sequence of Sequence #13: (SEQ ID NO: 26)

10 Genebank ID# NP_195540.2
 Positions 1-680 of NP_195540.

1 mttfstcaaf lslvvvlhav hvggaillesq aphrelkayr plqdnnlqev yassaaavhy
 61 dpdlkdvni atysdhygni rlgvrkmgdl spswvlenpa yqvsrktks qlviprdsfq
 15 121 ndtgmednas hsttnqtides enqfpnvdfa spaklrqil rqerrgqrtl elirqeketd
 181 eqmqeaaik smsfensvig kysiwrrdye spnadailkl mrdqiimaka yaniaksknv
 241 tnlyvflmqc cgenkrvig atsdadlpss aldqakamgh alsakdely dchelakkfr
 301 ailqsterkv dglkkkgftl iqlaaktfph plhclslqla adyfilgfne edavkedvsg
 361 kkledpslyh yaifsdnvla tsvvvnstvl nakepqrhvf hivtdklng amkmwfrina
 20 421 padatiqven indfkwlss ycsvlrqls arlkeyyfa nhpssisaga dnklyrnpky
 481 lsmlnhlrly lpevypklek ilfdddivv qkdlaplwei dmqgkvngav etckesfhrf
 541 dkylnfsnpk isenfdagac gwafgmnmdf lkewrkrnit giyhywqdl edrtlwklgs
 601 lppglitfyn ltyamdrswv vlglydpal nqtaienaav vhyngnykpw lglafakykp
 661 ywskyveydn pylrrcdine

Sequence #14 (SEQ ID NO: 27)

Gene name: At5g15470

GeneBank accession # for reference: NM_121551 GI:30685368

30 Nucleotide sequence of Sequence #14:
 Positions 1-1599 of CDS of NM_121551.

1 atgcagcttc acatatcgcc gagtatgaga agcattacga ttctgagcag caatgagttt
 61 attgacttga tgaagatcaa ggtcgagct cgtcacatct cttaccgaac tctctccac
 35 121 accatcttaa tctctgcttt ctgttgctt ttgttttca ttctaccgc tgtgttacc
 181 ctgaggggtg tcaacaaatg ctctccatt gattgtttag ggaggcggat aggtccacgt
 241 cttcttgga ggtagatga ttacagagaga ctacgttagag acitttataa aattctaaac
 301 gaagtaagca ctcaagaaat tccagatggt ttgaagcttc caaattctt tagtcaactt
 361 gttccgata tgaagaataa ccactatgat gcaaaaacat ttgctctgt gctgcgagcc
 40 421 atgatggaga agttgaacg tgatagagg gaatcgaaat ttgcagaact tatgaacaag
 481 cactttgcag caagttccat tcccaaaggc attcattgtc tctcttaag actgacagat
 541 gaatattcct ccaatgctca tgctcgaaga cagcttcctt caccagagtt tctccctgtt
 601 cttcagata atgcttacca ccacttatt ttgtccacgg acaatattt ggctgcctca
 661 gttgtggtct catccgctgt tcagtcattc tcaaaacccg agaaaattgt cttcacatc
 45 721 attacagaca agaaaaccta tgcgggtatg cattcatggt ttgcgcttaa ttctgttga
 781 ccagcaattg ttgaggttaa aggtgttcat cagttgact ggttgacgag agagaattg
 841 ccggttttgg aagctgtgga aagccataat ggtgtcaggg actattatca tgggaatcat
 901 gtcgctgggg caaacctcac cgaaacaact cctcgaacat ttgcttcaa attgcagtct
 961 agaagtccaa aatacatatc ttgtctcaac catcttagaa tataatatac agagctttt
 50 1021 ccgaacttgg acaaggtggt ttcttagac gatgatatag ttgtccaggg agacttaact

1081 ccactttggg atgttgacct cggtggttaag gtcaatgggg cagtagagac ttgcaggggt
 1141 gaagatgaat gggatgctc aaagcggtta aggaactact tcaatttctc tcacccgctc
 1201 atcgcaaagc atttagatcc tgaagaatgt gcttgggcat atggtatgaa tatcttcgat
 1261 ctacaagctt ggaggaaaac aaatatcaga gaaacgtatc actcttggtc tagagagaaat
 5 1321 ctaaagtcaa atctgacaat gtggaaactt ggaaccttgc ctctgctct tatcgcggtc
 1381 aagggtcacg tacacataat agactcgtca tggcatatgc taggattagg ctaccagagc
 1441 aagaccaaca tagaaaatgt gaagaaagca gcagtatcc actacaatgg gcagtcaaag
 1501 ccatggctgg agattgggtt cgagcatctg cggccattct ggaccaaata cgtcaactac
 1561 tcaaatgatt tcatcaagaa ctgtcacata ttggagtag

Amino Acid Sequence of Sequence #14: (SEQ ID NO: 28)

Genebank ID# NP_197051

Positions 1-532 of NP_197051.

1 mqlhispsmr sitisssnef idlmkikvaa rhisyrtlfh tililaflp fviltavvt
 61 legvnkcssi dclgrrigpr llgrvddser lardfykiln evstqeipdg lklpnsfsq
 121 vsdmknnhyd aktfalvira mmekferdmr eskfaelmnk hfaassipkg ihclslrltd
 181 eyssnaharr qlpspeflpv lsdnayhhfi lstdnilaas vvvssavqss skpekivfhi
 20 241 itdkktyagm hswfalnsva paivevkgvh qfdwltrenv pvleaveshn gvrddyhgnh
 301 vagantett prtfasklqs rspkyislln hlriyipelf pnldkvvfid ddivvqgdl
 361 plwdvdlggk vngavetcrq edewvmskrl rnyfnfshpl iakhldpeec awaygmni
 421 lqawrknir etyhswlren lksnltnmwl gltppaliaf kghvhiidss whmlglgyqs
 481 ktnienvkka avihyngqsk pwleigfehl rpfwtkyvny sndfiknchi le

Sequence #15 (SEQ ID NO: 29)

Gene name: At5g54690

GeneBank accession # for reference: NM_124850 GI:30696504

Nucleotide sequence of Sequence #15:

Positions 1-1608 of CDS of NM_124850.

1 atgcagttac atatatctcc gagcttgaga catgtgactg tggtcacagg gaaaggattg
 61 agagagtcca taaaagttaa ggttggttct agaagattct cttatcaaat ggtgttttac
 35 121 tctctactct tcttcacttt tcttctccga ttcgtctttg ttctctccac cgttgatact
 181 atcgacggcg atccctctcc ttgctcctct ctgcttgct tggggaaaag actaaagcca
 241 aagcttttag gaagaagggt tgattctggt aatgtccag aagctatgta ccaagtttta
 301 gaacagcctt taagcgaaca agaactcaaa ggaagatcag atatactca aacacttcaa
 361 gatttcatgt ctgaagtcaa aagaagcaaa tcagacgcaa gagaatttgc tcaaaagcta
 40 421 aaagaaatgg tgacattgat ggaacagaga acaagaacgg ctaagattca agagtattta
 481 tatcgacatg tcgcatcaag cagcataccg aaacaacttc actgtttagc tcttaaacta
 541 gccaacgaac actcgataaa cgcagcggcg cgtctccagc ttccagaagc tgagcttgct
 601 cctatgttgg tagacaacaa ctactttcac ttgtcttgg cttcagacaa tattcttgca
 661 gcttcggttg tggctaagtc gttggttcaa aatgctttaa gacctcataa gatcgttctt
 45 721 cacatcataa cggataggaa aacttatttc ccaatgcaag ctggttctc attgcatcct
 781 ctgtctccag caataattga ggtcaaggct ttgcatcatt tcgattggtt atcgaaagg
 841 aaagtacccg ttttgaagc tatggagaaa gatcagagag tgagggttca attcagaggt
 901 ggatcatcgg ttattgtggc taataacaaa gagaacccgg ttgtgttgc tgctaagtta
 961 caagcttca gccctaaata caactccttg atgaatcaca tccgtattca tctaccagag
 50 1021 ttgtttccaa gcttaaacaa ggttgtgttt ctagacgatg acattgtgat ccaaactgat

1081 cttcacctc ttgggacat tgacatgaat ggaaaagtaa atggagcagt ggaaacatgt
 1141 agaggagaag acaagttgt gatgtcaaag aagtcaaga gttacctcaa ctctcgaat
 1201 ccgacaattg ccaaaaactt caatccagag gaatgtgcat gggcttatgg aatgaatgt
 1261 ttcgacctag cggcttgag gaggactaac ataagctcca ctactatca ttggcttgac
 5 1321 gagaacttaa aatcagacct gagttgtgg cagctgggaa cttgcctcc tgggctgatt
 1381 gctttccacg gtcattgcca aaccatagat ccgttctggc atatgctgg tctcgatc
 1441 caagagacca cgagctatgc cgatctgaa agtgccgctg ttgtcattt caatggaaga
 1501 gctaagcctt ggctggatat agcatttctt catctacgct ctctctgggc taagtatct
 1561 gattcttctg acagatttat caagagctgt cacattagag catcatga

Amino Acid Sequence of Sequence #15: (SEQ ID NO: 30)

Genebank ID# NP_200280

Positions 1-535 of NM_200280.

1 mqlhispslr hvtvvtgkgl refikvkvg rfsyqmvfy slfftfllr fvfvlstvd
 61 idgdpsscsl lacgkrlkp klgrrvdsg nvpeamyqvl eqplseqelk grsdipqtlq
 121 dfmsevkrsk sdarefaqkl kemvtlmeqr trtakiqeyl yrhvasssip kqlhclalkl
 181 anehsinaaa rlqlpeaelv pmlvdnnyfh fvlasdnla asvvakslvq nalrphkivl
 241 hiitdrkyf pmqawfslhp lspaievka lhhfdwlskg kpvpleamek dqrvsqfrg
 301 gssvivannk enpvvvaakl qalspkynsl mnhirihlpe lfpslnkvvf ldddiviqtd
 361 lslplwidmn gkvngavetc rgedkfvmks kfkyslnfsn ptiaknfnpe ecawaygmnv
 421 fdlaawrrtn isstyhwld enlksdlslw qlgtlppgli afhghvqtid pfwhmlglgy
 481 qettsyadae saavvhfng akpwlidafp hlrplwakyl dssdrfiksc hiras

The nucleotide and amino acid sequences of the ten *GALAT-LIKE* gene family members are shown as follows.

Sequence #16 (SEQ ID NO:31)

Gene name: At1g02720

GeneBank accession # for reference: NM_100152, GI: 30678358

Nucleotide sequence of Sequence #16:

Positions 1-1086 of CDS of NM_100152.

1 atgcattgga ttacgagatt ctctgcttc ttctccgccc cattagccat gattctcctt
 61 tctccttcgc tccaatcctt ttctccgccc gcagctatcc gatcatctca cccctacgcc
 121 gacgaattca aacccaaca aaactccgat tactcctcct tcagagaatc tccaatgttc
 181 cgtaacgccc aacaatgcag atctccgccc gaagattccc gcgtctgtaa cctaattctc
 241 gtccacgtag ccatcactct cgacatcgat tacctccgtg gctcaatcgc agccgtcaat
 301 tcgatcctcc agcactcaat gtgccctcaa agcgtcttct tccacttctc cgtctcctcc
 361 gagtctcaaa acctagaatc tctgattcgt tctactttcc ccaaatgac gaattcaaaa
 421 attactatt ttgcccctga gaccgtacag tcttgattt catcttccgt gagacaagcc
 481 ctagagcaac cgttgaatta cgccagaaat tacttgccgg atctgctcga gccttgctgt
 541 aagcgagtca tctacttggg ttccgattct gtcgtcgtcg atgatatcgt caagctttgg
 601 aaaacggggt taggccagag aacaatcgga gtcgccgagt attgtcacgc gaatttcacg
 661 aaatacttca ccggagggtt ttggtcagat aagaggttta acgggacgtt caaagggagg
 721 aacccttgtt acttcaatac tgggtgaatg gtgattgatt tgaagaagt gagacaatt

781 aggttcacga aacgaattga gaaatggatg gagattcaga agatagagag gatttatgag
 841 ctgggttctc ttctccgtt tcttctgga ttgctggc atgtagctcc gatttcacat
 901 cggtggaatc aacatgggct tgggtggat aatgttagag gtagtgccg tgatttgc
 961 tctggtcctg tgagtttct tactgtgca gtagtgga agccatggt aagactcgat
 5 1021 tccaagcttc catgtcctt agacacattg tgggcacct atgatttga taaacactcc
 1081 cattga

10 Amino Acid Sequence of Sequence #16: (SEQ ID NO: 32)
 Genebank ID# NP_171772
 Positions 1-361.

15 1 mhwitrfsaf fsaalamill spslqsfspa aairsshpya defkpqqnsd yssfrespmf
 61 rnaeqcrssg edsgvcnpnl vhwaitldid ylrsgiaavn silqhsmpcp svffhflvss
 121 esqnleslir stfpkltnlk iyyfapetvq slisssvrqa leqplnyam yladllepcv
 181 krviyldsdl vvdddivklw ktglgqrtig apeychanft kyftggfwsd krngtfgkr
 241 npcyfntgvm vidlkkwrqf rtkriekwm eiqkieriye lgsppfliv faghvapish
 20 301 rwnqhglggd nvrsgcrdlh sgpvsllhws gsgkpwrlrd skipcpldtl wapydlykhs
 361 h

Sequence #17 (SEQ ID NO:33)

Gene name: At1g13250

25 GeneBank accession # for reference: NM_101196, GI:30683194
 Nucleotide sequence of Sequence #17:
 Positions 1-1038 of CDS of NM_101196.

30 1 atgtcttctc tgcgtttgcg ttatgtctt cttctactct tacctatcac aattagctgc
 61 gtcacagtca ctctactga cctccccgcg ttctgtgaag ctccggcgtt tcgaaacggc
 121 agagaatgct ccaaaacgac atggatacct tcggatcacg aacacaacct atcaatcatc
 181 cacatcgcta tgactctcga cgcaatttac ctccgtggct cagtcgccgg cgtcttctcc
 241 gttctccaac acgcttcttg tctgaaaac atcgtttcc acttcacgc cactcaccgt
 35 301 cgcagcgccg atctccgccg cataatctcc tcaacattcc catacctaac ctaccacatt
 361 taccatttg accctaacct cgtccgcagc aaaatatctt cctctattcg tcgtgcttta
 421 gaccaaccgt taaactacgc tcggatctac ctgcgccgac tcttcccaat cgccgtccgc
 481 cgcgtaatct acttcgactc cgatctcgta gtcgtcgatg acgtggctaa actctggaga
 541 atcgatctac gtcggcacgt cgtcggagct ccggagtact gtcacgcgaa ttactaactc
 40 601 tacttcaact caagattctg gtcgagtcaa ggttacaat cggcgttgaa agataggaaa
 661 ccgtgttatt tcaacaccgg agtgatggtg attgatctcg gaaaatggag agaaaggaga
 721 gtcacgggta agctagagac atggatgagg attcaaaaac gacatcgat ttacgaattg
 781 ggatctttgc ctccgtttct gctcgtttc gccggagatg ttgagccggt ggagcatagg
 841 tggaatcagc atggtcttgg tggatgataac ttggaaggac ttgcccga ttgcatcca
 45 901 ggtccgggta gtttgtgca ttggagcggg aaagggaac catggctaag gcttgactcg
 961 agacgaccgt gtccgttga ttcgttatg gtccttatg attgtttcg ttattaccg
 1021 ttgactctg atagctga

Amino Acid Sequence of Sequence #17: (SEQ ID NO: 34)
 Genebank ID# NP_563925
 Positions 1-345.

```

5      1 msslrllrl lllpitisc vvtltldipa freapafrng recskttwip sdhehnpsii
      61 hiamtldaiy lrgsvagvfs vlqhascpen ivfhfiathr rsadlriis stfpyltyhi
      121 yhfdpnlvrs kisssirral dqplnyariy ladllpiavr rviyfdsdv vvddvklwr
      181 idlrrhvga peychanftn yftsrwssq gyksalkdrk pcyfntgvmv idlgkwerrr
      241 vtvkletwmr iqkrhriel gslppflvf agdvepvehr wnqhglggdn leglcrnlhp
10     301 gpvsllhwsg kgkpwrlids rrpcpldslw apydlfrysp lisd
  
```

Sequence #18 (SEQ ID NO:35)

Gene name: At1g19300

GeneBank accession # for reference: NM_101787, GI:30686302

15 Nucleotide sequence of Sequence #18:
 Positions 1-1056 of CDS of NM_101787.

```

      1 atgtccaac atcttctct tctcattctc ctctcgctac ttcttctca taaaccatt
      61 tccgccacta caattattca aaaattcaaa gaagccccac agttttacaa ttctgcagat
20     121 tcccccttaa tcgatgactc cgagtcgac gatgacgtgg tcgcaaacc aatctctgc
      181 tcacgtcgag ctgtccacgt ggcatgaca ctgacgccg cctacattcg tggctcagtc
      241 gccgctgttc tctccgtcct ccaacactct tctgtcctg aaaacattgt ttccacttc
      301 gtcgcctctg ctccgccga cgcttcttc ttacgagcca ccatactctc ctcttccct
      361 tacctgatt tcaccgtcta cgtcttcaac gtctcctcg tctctcgct tatctcctc
25     421 tctatccgct cgcactaga ctgtcctta aactacgcaa gaagctacct cgccgatctc
      481 ctccctcct cgtccgccg cgtcgtctac ctgactccg atctgatcct cgtcgacgac
      541 atagcaaaac tcgccccac agatctggc cgtgattcag tctcgccgc gccggaatac
      601 tgcaacgcca atttcactc atacttaca tcaaccttct ggtctaatac gactctctc
      661 ttaacctcg ccgatcgga agcatgctac ttcaacactg gagtcattgt gatcgatct
30     721 tcccggtggc gcgaaggcg gtacacgtca cgcatcgaag agtggatggc gatgcaaaag
      781 agaatgagaa ttacgagct tggttcgta ccaccgttt tattggttt tgcgggttg
      841 attaaaccgg ttaatcatc gtggaacaa cacggttag gagtgataa tttagagga
      901 ctgttagag atctccatc tggtcggtg agtctgtgc attggagtgg gaaaggtaag
      961 ccatgggcta ggcttgatgc tggtcggcct tgcctttag acgcgcttg ggctccgtat
35     1021 gatcttctc aaacgccgt cgcttggat tcttga
  
```

Amino Acid Sequence of Sequence #18: (SEQ ID NO: 36)
 Genebank ID# NP_564077
 Positions 1-351.

```

40     1 msqhlillil lslllhkpi sattiiqkfk eapqfynsad cpliddsed ddvvakpifc
      61 srravhvamt ldaayirgsv aavslvlqhs scpenivfhf vasasadass lratissf
      121 yldftvyvfn vssvsliss sirsaldcpl nyarsyladi lppcvrrvy lsdliivdd
      181 iaklaatdlg rdsvlaapey cnanftsyt stfwsnptls ltfadrkacy fntgvmvidl
45     241 srwregayts rieewmamqk rmriyelgsi ppflvfagl ikpvnhrwnq hglggdnfrg
      301 lcrdlhpgpv slhwsgkgk pwarldagrp cpdlalwapy dllqtpfald s
  
```

Sequence #19 (SEQ ID NO:37)

Gene name: At1g24170

GeneBank accession # for reference: NM_102263, GI:30688765

5 Nucleotide sequence of Sequence #19:

Positions 1-1182 of CDS of NM_102263.

```

      1 atgtcgtcgc gttttcttt gacggtggtg tgttgattg ctctgttacc gtttgtgtt
      61 ggtatacggg tgattccggc gaggatcacg agtgcgggtg atggcggcgg cggaggagggt
10    121 aataatgggt ttagtaaact tgggccgttt atggaagctc cggagtatag aaacggcaag
      181 gagtgtgtat ctcatcagc gaacagagag aacttcgtgt cgtcttcttc tagttcta
      241 gatccttcgc ttgtcacat cgctatgact ttgactcag agtatctccg tggatcaatc
      301 gcagccgttc attctgttct tcgccacgcg tctgtccag agaacgtctt ctccatttc
      361 atcgctgctg agtttgactc tgcgagtcct cgtgttctga gtcaactcgt gaggtcgact
15    421 ttccctcgt tgaacttta agtctacatt ttagggaag atacgggat caatctcata
      481 tcttctcga ttagactagc ttggagaat cgttgaact atgctcggaa ctatctcgga
      541 gatattcttg atcgaagtgt tgaacgagtc attatcttg actcggatgt tataactgtg
      601 gatgatatca caaagctttg gaacacgggt ttgaccgggt cacgagtcac cggagctccg
      661 gagtattgtc acgcaactt cactcagat ttacttccg gggtctgggc agaccgggt
20    721 ttaccgggtc taatctcggg tcaaaagcct tgctattca acacaggagt gatgggtgat
      781 gatcttgta gatggagaga aggaattac agagagaagt tagagcaatg gatgcaattg
      841 cagaagaaga tgagaatcta cgatcttga tcattaccac cgttctttt ggtgtttgcg
      901 ggtaatgtg aagctattga tcatagatgg aaccaacatg gtttaggagg agacaatata
      961 cgaggaagtt gtcggtcatt gcatcctggc cctgtgagct tgttgcatg gagtggtaaa
25   1021 ggtaagccat gggtagact tgatgagaag aggcctgtc cgttgatca tcttgggag
      1081 ccatatgatt tgtataagca taagattgag agagctaaag atcagtctc gcttggggtt
      1141 gcttctctgt cggagttgac tgatgattca agcttctgt ga

```

30 Amino Acid Sequence of Sequence #19: (SEQ ID NO: 38)

Genebank ID# NP_173827

Positions 1-393.

```

      1 mssrfsltvv clialpfvv girliparit svgdgggggg nngfsklgpf meapeyrngk
35    61 ecvsssvnre nfvsssssn dpslvhiamt ldseylrgsi aavhsvlrha scpenvfhhf
      121 iaafdsasp rvlsqlvrst fpslnfkvyi fredtvinli sssirlalen plnyarnylg
      181 dildrsverv iyldsdivtv ddiiklwntv ltgsrvigap eychanftqy ftsgfwsdpa
      241 lpglisgqkp cyfntgvmvm dlvrwregny rekleqwmql qkkmriydlg slppfllvfa
      301 gnveaidhrw nqhglggdni rgscrslhpg pvsllhwsbk gkpwwrldk rcppldhlwe
40    361 pydlykhkie rakdqslgf aslseltds sfl

```

Sequence #20 (SEQ ID NO:39)

Gene name: At1g70090

GeneBank accession # for reference: NM_105677, GI:30697975

5 Nucleotide sequence of Sequence #20:

Positions 1-1173 of CDS of NM_105677.

```

1 atgCGgttgc gtttccgat gaaatctgcc gtttagcgt ttgctatctt tctgggtgtt
61 attccactgt ttccgctcg tatacggatg attccgggaa gactcaccgc cgtatccgcc
10 121 accgtcggaa atggcttga tctggggtcg ttcgtggaag ctccggagta cagaaacggc
181 aaggagtgcg tgtcctaac gtgaacaga gaaaacttcg tgcgtcttg cgacgcttcg
241 ttagtcatg tagctatgac gctgactcg gactacttac gtggctcaat cgcagccgta
301 cattcaatgc tccgccacgc gtcgtgtcca gaaaacgtct tcttccatct catcgctgca
361 gagtttgacc cggcgagtcc acgcgttctg agtcaactcg tccgatctac ttcccgctcg
15 421 ctaaacttca aagtctacat ttccgggaa gatacgggta tcaaccttat ctcttctca
481 atcagacaag ctttagagaa tccattgaac tatgctcgga actacctcgg agatattctt
541 gatccatgcg tagacagagt catttacctt gactcggaca tcatcgtcgt cgatgacata
601 acaaagcttt ggaacacgag ttgacaggg tcaagaatca tccgagctcc ggagtattgt
661 cacgctaact tcacaaagta ctacacttca ggttctggt ccgacccggc ttaccgggt
20 721 ttcttctcgg gtcgaaagcc ttgtatttc aacacgggtg tgatgggat ggatctagtt
781 agatggagag aaggaaacta cagagaaaag ctgaaactt ggatgcagat acagaagaag
841 aagagaatct acgattggg ttcttgcct ccgttcttc ttgtcttcgc agggaacgtt
901 gaagcaattg atcatagggt gaaccaacat ggttaggag gagacaatgt acgaggaagt
961 ttaggtctt tgcataaagg accagttagt ttgtgcatt ggagtggtaa aggtaagcca
25 1021 tgggtgagac ttgatgagaa gagaccgtgt ccgttgatc attatggga accgatgat
1081 ttatatgagc ataagattga aagagctaaa gatcagtctt tgttcgggtt ctctctttg
1141 tctgagttaa cagaagattc aagcttttc tga

```

30

Amino Acid Sequence of Sequence #20: (SEQ ID NO: 40)

Genebank ID# NP_564983

Positions 1-390.

35

```

1 mrlrfpmksa vlafailvf iplfsvgirm ipgrltavsa tvngfdlgs fveapeyrng
61 kecvsqslnr enfvsscdas lvhvamtlds eylrgsiaav hsmrlhascp envffhliaa
121 efdpasprvl sqlvrstfps lnfkvyifre dtvinlsss irqalenpln yarnylgdil
181 dpcvdrviyl dsdiivddi tklwntsltg sriigapeyc hanftkyfts gfwsdpalpg
40 241 ffsgrkpcyf ntgvvmvdlv rwregnyrek letwmqiqkk kriydlgslp pflilfagnv
301 eaidhrwnqh glggdnvrgs crslhkgpvs llhwsgkgkp wvrldkrpc pldhlwepyd
361 lyehkierak dqlfgfssl seltedssff

```


Sequence #21 (SEQ ID NO:41)

Gene name: At3g06260

GeneBank accession # for reference: NM_111501, GI:18397517

5 Nucleotide sequence of Sequence #21:

Positions 1-1056 of CDS of NM_111501.

```

1 atggcctcaa ggagcctctc ctatacaca ctcctaggcc tctgtcctt tatactcctc
61 ttggtcacia ccaccactat ggcggttcgt gttggagtca ttctcataa gccttctgct
10 121 ccaactcttc ctgttttcag agaagccccg gcttttcgaa acggtgatca atgcgggact
181 cgtgaggctg atcagattca tatcgccatg actctcgaca caaactacct ccgtggcaca
241 atggctgccg tttgtctct cttcaacat tccacttgcc ctgaaaacct ctctttcat
301 ttctgtccc ttctcattt cgaaaacgac ctttcacca gcatcaaata aacctttcct
361 tacctaaact tcaagattta tcagtttgat ccaaacctcg tccgcagcaa gatatcgaaa
15 421 tccatcaggc aagcccttga tcagcctctt aactacgcaa gaatctacct cgcgatatc
481 atccctagca gcgttgacag gatcatctac ttagactcag acctcgttgt ggtagacgac
541 atagagaagc tgtggcatgt ggagatggaa ggtaaagtgg tggctgctcc cgagtactgc
601 cagcgaact tcacccatta ttcaacaaga actttctggt cagaccgggt attggtcaaa
661 gttctgaag gaaaacgtcc gtgttattc aacacagggg tgatggttgt ggatgtaaac
20 721 aaatggagga aaggaatgta tacacagaag gtagaagagt ggatgacaat tcagaagcag
781 aagaggatat accatttggg atcattacct cgtttctgc tgatatcgc cggatgata
841 aaagcggtta atcatagggt gaaccagcat ggtctaggag gtgataattt cgaaggaaga
901 ttagaacgt tgcattccgg accgataagt cttcttact ggagtggaaa agggaagcca
961 tggtaagac tagattcaag gaagccttgt atcgttgatc atctatgggc accgtatgat
25 1021 ctgtaccgtt catcaagaca ttcattagaa gagtag

```

Amino Acid Sequence of Sequence #21: (SEQ ID NO: 42)

30 Genebank ID# NP_187277

Positions 1-351.

```

1 masrslytq llgllsfill lvtttmavr vgvilhkpsa ptlpvfreap afrngdqcg
61 readqihiam tldtnylrgt maavlsllqh stcpnlshf flslphfend lftsikstfp
35 121 ylnfkiyqfd pnlvrskisk sirqaldqpl nyariyladi ipssvdriiy ldsdlvvdd
181 ieklwhveme gkvvaapeyc hanfthyftr tfwsdpvlvk vlegkrpcyf ntgvmvvdvn
241 kwrkgmytqk veewmtiqkq kriyhlslp pfllifagdi kavnhwrwnqh glggdnfegr
301 crtllhpgpis llhwsqgkpk wlrldsrkpc ivdhlwapyd lyrssrshle e

```

Sequence #22 (SEQ ID NO:43)

Gene name: At3g28340

GeneBank accession # for reference: NM_113753, GI:30689155

5 Nucleotide sequence of Sequence #22:

Positions 1-1098 of CDS of NM_113753.

```

1 atgatgtctg gttcaagatt agcctctaga ctaataataa tcttctcaat aatctccaca
61 tcttcttca cgttgaatc gattcgacta ttccctgatt cattcgacga tgcattctca
10 121 gatttaatgg aagctccagc atataaaaac ggtcttgatt gctctgtttt agccaaaaac
181 agactcttgt tagcttgatg tccatcagct gttcatatag ctatgactct agatccagct
241 tacttgctg gcacggatc tgcagtacat tccatcctca aacacacttc ttgccctgaa
301 aacatcttct tccacttcat tgcttcgggt acaagtcagg gtccctcgc caagacccta
361 tctctgttt ttcttcttt gagttcaaa gtctatacct ttgaagaaac cacggtcaag
15 421 aatctaattc ctcttctat aagacaagct ctgtagatc cttgaatta cgcaagaagc
481 tacttatccg agattcttc ttcgtgtgt agtcgagtga tttatctcga ttcggtatgt
541 attgtggtcg atgatattca gaaactatgg aagatttctt tatccgggtc aagaacaatc
601 ggtgcaccag agtatgcca cgcaaattc accaaatact tcacagatag ttctgggtcc
661 gatcaaaaac tctcgagtgt cttcgattcc aagactcctt gttatttcaa cacaggagtg
20 721 atggttatcg atttagagcg atggagagaa ggagattaca cgagaaagat cgaaaactgg
781 atgaagattc agaaagaaga taagagaatc tacgaattgg gttctttacc accgtttctt
841 ctagtgtttg gtggtgatat tgaagctatt gatcatcaat ggaaccaaca cggctcgggt
901 ggagacaaca ttgtgagtag ttgtagatct ttgcatcctg gtccgggttag ttgatacat
961 tggagtggta aagggaagcc atgggttagg ctgatgatg gtaagccttg tccaattgat
25 1021 tatcttggg ctcttatga tctcacaag tcacagaggc agtatcttca atacaatcaa
1081 gagttagaaa ttcttga

```

30 Amino Acid Sequence of Sequence #22: (SEQ ID NO: 44)

Genebank ID# NP_189474

Positions 1-365.

```

35 1 mmsgsrslasr liifsiist sftvesirl fpdsfddass dlmeapayqn glcdsvlakn
61 rllacdpsa vhamtldpa ylrvtasvnh silkhtscpe niffhfiasg tsqgslakti
121 ssvfplsfsk vyfteetvk nlissirqa ldspinyars ylseilsscv srviyldsdv
181 ivvddiqklw kislgsrti gapeychanf tkyftdsfws dqklssvfdk ktpcyfntgv
241 mvidlenwre gdytrkienw mkiqkedkri yelgslppfl lvfggdieai dhqwnqhglg
40 301 gdnivsscrs lhpqpvslh wsgkgkpwr lddgkpcpid ylwapydlhk sqrylqynq
361 eleil

```

Sequence #23 (SEQ ID NO:45)

45 Gene name: At3g50760

GeneBank accession # for reference: NM_114936, GI:18409176

Nucleotide sequence of Sequence #23:

Positions 1-1026 of CDS of NM_114936.

50 1 atgcactcga agtttatatt atatctcagc atcctcgccg tattcaccgt ctctttcgcc

61 ggcggcgaga gattcaaaga agctccaaag ttctcaact ccccgagtg tctaaccatc
 121 gaaaacgatg aagatttcgt ttgtcagac aaagccatcc acgtggcaat gaccttagac
 181 acagcttacc tccgtggctc aatggccgtg attctctccg tctccaaca ctctcttgt
 241 cctcaaaaca ttgtttcca ctctgtact tcaaaacaaa gccaccgact ccaaaactac
 5 301 gtcgttgctt ctttcccta ctgaaattc cgaatttacc ctacgacgt agccgccatc
 361 tccggcctca tctcaacctc catccgctcc gcgctagact ctccgctaaa ctacgcaaga
 421 aactacctcg ccgacattct tcccacgtgc ctctacgtg tcgtatacct agactcagat
 481 ctcatactcg tcgatgacat ctcaaagctc ttctcactc acatccctac cgacgtcgtt
 541 ttgcccgcgc ctgagtactg caacgcaaac ttacgactt actttactcc gacgttttg
 10 601 tcaaaccctt ctctctccat cacactatcc ctcaaccgcc gtgctacacc gtgttacttc
 661 aacaccggag tgatgggtcat cgagttaaag aatggcgag aaggagatta cagaggaag
 721 atcatagagt ggatggagtt acaaaaacgg ataagaatct acgagtagg ctcttacc
 781 ccgtttttac ttgtcttcgc cggaacata gctccggtag atcaccggtg gaaccaacac
 841 ggtttaggag gagataattt tagaggactg tctcgagatt tgcattccagg tccagtgagt
 15 901 ttgtgcatt ggagtgggaa agggaagcca tgggtaagg tagatgatgg tcgacctgac
 961 ccgttgatg cacttgggt tccatgatg ttgttagagt cagggttga cctatcgag
 1021 agttaa

20 Amino Acid Sequence of Sequence #23: (SEQ ID NO: 46)
 Genebank ID# NP_190645
 Positions 1-341.

1 mhskfilyls ilavftvsfa ggerfkeapk ffnspecti endedfvcsd kaihvamtld
 25 61 taylrgsmav ilsvlqhssc pqnivfhfv skqshrlqny vvasfpylkf riypdvaa
 121 sglstirs aldspnyar nyladilptc lsrvyldsd liivddiskl fsthptdv
 181 laapeycnan ftyftptfw snpslsitls lnrratpcyf ntgvmmielk kwregdytrk
 241 iiewmelqkr iriyelgslp pflvfvagni apvdhrwnqh glgdnfrgl crdlhpgpvs
 301 llhwsqgkqp wvrlldgrpc pldalwvpyd llesrfdlie s

Sequence #24 (SEQ ID NO:47)

Gene name: At3g62660

GeneBank accession # for reference: NM_116131, GI:30695642

35 Nucleotide sequence of Sequence #24:
 Positions 1-1086 of CDS of NM_116131.

1 atgctttgga tcatgagatt ctccggttta ttctccgccg ctttggttat catcgtcctc
 40 61 tctccttctc tccaatcgtt tctccagct gaagctatca gatccttca tctcgacgt
 121 tacctccgtt tcccctctc cgatccaccg ccgcatagat tctcctcag aaaagctcct
 181 gtttccgca atgccgccga ttgcgccgcc gcagatatcg attccggcgt ctgtaaccct
 241 tcttggtcc acgtcgcat tactctcgat tctgagtacc tgcgtggctc aatcgccgcc
 301 gttcattcga ttctcaagca ctgctcgtgt cccgagagcg tcttctcca ttctctgctc
 45 361 tccgagactg acctagaatc ctgattcgt tgcactttc ccgaattgaa attaaagggt
 421 tactacttcg atccggagat tctacggacg ctgatctcaa cctccgtgag acaagcgctc
 481 gagcagccgt tgaattacgc tagaaattac ctgctgacc ttctcgagcc ttgcgtcgt
 541 cgcgtgatct acctagattc cgatctaata gtcgtcgacg acatcgcaaa gctctggatg
 601 acgaaactgg gatcgaaaac gatcggagct cccgagtact gtcacgcgaa cttcacaag
 50 661 tattcacac cggcgttctg gtccgacgag aggttctccg gagcttctc cgggaggaaa

721 ccgtgctact tcaacacggg agtgatggtg atggatctag agagatggag ggcgctaggg
 781 tacacggagg tgatagagaa atggatggag attcagaaga gtgataggat ttacgagctg
 841 ggatcattgc cgccgttctt gttggtgttc gccggagaag tagctccgat agagcatcgg
 901 tggaaaccagc atgggcttgg tggagataac gtgagaggaa gctgtagaga ttacatccc
 5 961 ggtccgggta gcttgcttca ttggtccggt agtggtaaac cgtggtttcg gttagattcg
 1021 agacggcctt gtccacttga tactcttgg gcacctatg attgtatgg acactactct
 1081 cgctga

10 Amino Acid Sequence of Sequence #24: (SEQ ID NO: 48)
 Genebank ID# NP_191825
 Positions 1-361.

1 mlwimrfsgl fsaalviivl spslqsfppa eairsshlda ylrpssdpp phrfsfrkap
 15 61 vfrnaadcaa adidsgvcnp slvhvaitld feylrgsaa vhsilkhssc pesvffhflv
 121 setdleslir stfpelklkv yyfdpeivrt listsvrqal eqplnyarny ladllepcvr
 181 rviyldsdli vvddiaklwm tklgsktiga peychanftk yftpafwsde rfsgafsgrk
 241 pcyfntgvmv mdlerrwrv yteviekwme iqksdriyel gslppflvf agevapiehr
 301 wnqhlggdn vrgscrdlhp gpvsllhws gskpwrlds rrpcpldtlw apydylyghys
 20 361 r

Sequence #25 (SEQ ID NO:49)

Gene name: At4g02130

25 GeneBank accession # for reference: NM_116445, GI:18411845
 Nucleotide sequence of Sequence #25:
 Positions 1-1041 of CDS of NM_116445.

1 atgctttgga taacgagatt tgctggatta ttctccgccg cgatggcagt gatcgtgta
 30 61 tctccgtcgc ttacgtcatt tctccggcg gcggcaatcc gttctctcc atcaccgac
 121 ttcagaaaag ctccagcggg gttcaacaac ggcgacgaat gtctctctc cggcggcgtc
 181 tgcaatccgt cgttggtcca cgtggcgatc acgttagacg tagagtacct gcgtggctca
 241 atcgagccg ttaactgat ccttcagcac tcggtgtgtc cagagagcgt cttctccac
 301 ttcacgccc tctccgagga acaaacctg ttggagtcgc tggtagatc ggtttcccg
 35 361 agactgaaat tcaatatta cgatttgcc cctgagacag ttcgtgggtt gatttctct
 421 tccgtgagac aagctctcga gcagcctctg aactacgcta gaagctactt agcggatctg
 481 ctggagcctt gtgtaaccg tgcataatc ttgattcgg atctgtcgt cgtcgatgac
 541 atcgctaagc ttggaaaac tagcctaggc tcgaggataa tcggagctcc ggagtattgt
 601 cacgcgaatt tcacgaaata cttaccgga ggattctggt cggaggagag attctccgg
 40 661 accttagag ggaggaagcc atgttactc aacacagggt tgatggtgat agatctaag
 721 aaatggagaa gaggtgggta cacgaaacgt atcgagaaat ggatggagat tcagagaaga
 781 gagaggattt acgaactagg ctgcgttcca ccgtttctc tagtttctc cggtcacgtg
 841 gctcccatct ctcaccggtg gaaccagcat ggacttggtg gtgacaatgt tagaggtagc
 901 tgtcgtgatt tgcattcgtg tctgtgagt ttgctgcat ggtctggtg tggcaagccc
 45 961 tggataagac tcgattccaa acggcctgt cccttagacg cattatggac gccttacgac
 1021 ttgatcgcac attcgattg a

Amino Acid Sequence of Sequence #25: (SEQ ID NO: 50)
 Genebank ID# NP_192122
 Positions 1-346.

```

5      1 mlwitrfaql fsaamavivl spslqsfppa aairstpspi frkapavfnn gdeclssggv
      61 cnpslvhvai tldveylrgs iaavnsilqh svcpesvffh fiavseetnl leslvrsvfp
      121 rlkfnidyfa petvrgliss svrqaleqpl nyarsyladl lepcvnrviv ldsdlvvvdd
      181 iaklwktslg sriigapeyc hanftkyftg gfwseerfsg tfrgrkpcyf ntgvmmvidlk
      241 kwrrggytkr iekwmeiqrr eriyelgslp pflvfsgghv apishrwnqh glggdnvrgs
10     301 crdlhpgpvs llhwsgsgkp wirlskrpc pldalwtpyd lyrhsh
  
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Those skilled in the art will appreciate that the invention described herein is susceptible to variations and modifications other than those specifically described. It is to be understood that the invention includes all such variations and modifications. The invention also includes all of the steps, features, compositions and compounds referred to or indicated in this specification, individually or collectively, and any and all combinations of any two or more of said steps or features.

The amino acids which occur in the various amino acid sequences referred to in the specification have their usual three- and one-letter abbreviations routinely used in the art: A, Ala, Alanine; C, Cys, Cysteine; D, Asp, Aspartic Acid; E, Glu, Glutamic Acid; F, Phe, Phenylalanine; G, Gly, Glycine; H, His, Histidine; I, Ile, Isoleucine; K, Lys, Lysine; L, Leu, Leucine; M, Met, Methionine; N, Asn, Asparagine; P, Pro, Proline; Q, Gln, Glutamine; R, Arg, Arginine; S, Ser, Serine; T, Thr, Threonine; V, Val, Valine; W, Try, Tryptophan; Y, Tyr, Tyrosine.

A protein is considered an isolated protein if it is a protein isolated from the plant, or from a host cell in which it is recombinantly produced. It can be purified or it can simply be free of other proteins and biological materials with which it is associated in nature.

An isolated nucleic acid is a nucleic acid the structure of which is not identical to that of any naturally occurring nucleic acid or to that of any fragment of a naturally occurring genomic nucleic acid spanning more than three separate genes. The term therefore covers, for example, (a) a DNA which has the sequence

of part of a naturally occurring genomic DNA molecule but is not flanked by both of the coding or noncoding sequences that flank that part of the molecule in the genome of the organism in which it naturally occurs; (b) a nucleic acid incorporated into a vector or into the genomic DNA of a prokaryote or eukaryote in a manner
5 such that the resulting molecule is not identical to any naturally occurring vector or genomic DNA; (c) a separate molecule such as a cDNA, a genomic fragment, a fragment produced by polymerase chain reaction (PCR), or a restriction fragment; and (d) a recombinant nucleotide sequence that is part of a hybrid gene, i.e., a gene encoding a fusion protein. Specifically excluded from this definition are
10 nucleic acids present in mixtures of (i) DNA molecules, (ii) transformed or transfected cells, and (iii) cell clones, e.g., as these occur in a DNA library such as a cDNA or genomic DNA library.

As used herein expression directed by a particular sequence is the
15 transcription of an associated downstream sequence. If appropriate and desired for the associated sequence, there the term expression also encompasses translation (protein synthesis) of the transcribed RNA. When expression of a sequence of interest is "up-regulated," the expression is increased. With reference to up-regulation of expression of a sequence of interest operably linked to a
20 transcription regulatory sequence, expression is increased.

In the present context, a promoter is a DNA region which includes sequences sufficient to cause transcription of an associated (downstream) sequence. The promoter may be regulated, i.e., not constitutively acting to cause
25 transcription of the associated sequence. If inducible, there are sequences present which mediate regulation of expression so that the associated sequence is transcribed only when an inducer molecule is present in the medium in or on which the organism is cultivated. In the present context, a transcription regulatory sequence includes a promoter sequence and can further include cis-active
30 sequences for regulated expression of an associated sequence in response to environmental signals.

One DNA portion or sequence is downstream of second DNA portion or sequence when it is located 3' of the second sequence. One DNA portion or

sequence is upstream of a second DNA portion or sequence when it is located 5' of that sequence.

One DNA molecule or sequence and another are heterologous to another if the two are not derived from the same ultimate natural source. The sequences may be natural sequences, or at least one sequence can be designed by man, as in the case of a multiple cloning site region. The two sequences can be derived from two different species or one sequence can be produced by chemical synthesis provided that the nucleotide sequence of the synthesized portion was not derived from the same organism as the other sequence.

An isolated or substantially pure nucleic acid molecule or polynucleotide is a polynucleotide which is substantially separated from other polynucleotide sequences which naturally accompany a native transcription regulatory sequence. The term embraces a polynucleotide sequence which has been removed from its naturally occurring environment, and includes recombinant or cloned DNA isolates, chemically synthesized analogues and analogues biologically synthesized by heterologous systems.

A polynucleotide is said to encode a polypeptide if, in its native state or when manipulated by methods known to those skilled in the art, it can be transcribed and/or translated to produce the polypeptide or a fragment thereof. The anti-sense strand of such a polynucleotide is also said to encode the sequence.

A nucleotide sequence is operably linked when it is placed into a functional relationship with another nucleotide sequence. For instance, a promoter is operably linked to a coding sequence if the promoter effects its transcription or expression. Generally, operably linked means that the sequences being linked are contiguous and, where necessary to join two protein coding regions, contiguous and in reading frame. However, it is well known that certain genetic elements, such as enhancers, may be operably linked even at a distance, i.e., even if not contiguous.

The term recombinant polynucleotide refers to a polynucleotide which is made by the combination of two otherwise separated segments of sequence accomplished by the artificial manipulation of isolated segments of polynucleotides by genetic engineering techniques or by chemical synthesis. In so doing one may join together polynucleotide segments of desired functions to generate a desired combination of functions.

Polynucleotide probes include an isolated polynucleotide attached to a label or reporter molecule and may be used to identify and isolate other sequences, for example, those from other species or other strains. Probes comprising synthetic oligonucleotides or other polynucleotides may be derived from naturally occurring or recombinant single or double stranded nucleic acids or be chemically synthesized. Polynucleotide probes may be labeled by any of the methods known in the art, e.g., random hexamer labeling, nick translation, or the Klenow fill-in reaction.

Large amounts of the polynucleotides may be produced by replication in a suitable host cell. Natural or synthetic DNA fragments coding for a protein of interest are incorporated into recombinant polynucleotide constructs, typically DNA constructs, capable of introduction into and replication in a prokaryotic or eukaryotic cell. Usually the construct is suitable for replication in a unicellular host, such as *A. pullulans* or a bacterium, but a multicellular eukaryotic host may also be appropriate, with or without integration within the genome of the host cell. Commonly used prokaryotic hosts include strains of *Escherichia coli*, although other prokaryotes, such as *Bacillus subtilis* or a pseudomonad, may also be used. Eukaryotic host cells include yeast, filamentous fungi, plant, insect, amphibian, mammalian and avian species. Such factors as ease of manipulation, ability to appropriately glycosylate expressed proteins, degree and control of protein expression, ease of purification of expressed proteins away from cellular contaminants or other factors influence the choice of the host cell.

The polynucleotides may also be produced by chemical synthesis, e.g., by the phosphoramidite method described by Beaucage and Caruthers (1981) *Tetra. Letts.*, 22: 1859-1862 or the triester method according to Matteuci *et al.* (1981) *J.*

Am. Chem. Soc., 103:3185, and may be performed on commercial automated oligonucleotide synthesizers. A double-stranded fragment may be obtained from the single stranded product of chemical synthesis either by synthesizing the complementary strand and annealing the strand together under appropriate conditions or by adding the complementary strand using DNA polymerase with an appropriate primer sequence.

DNA constructs prepared for introduction into a prokaryotic or eukaryotic host will typically comprise a replication system (i.e. vector) recognized by the host, including the intended DNA fragment encoding the desired polypeptide, and will preferably also include transcription and translational initiation regulatory sequences operably linked to the polypeptide-encoding segment. Expression systems (expression vectors) may include, for example, an origin of replication or autonomously replicating sequence (ARS) and expression control sequences, a promoter, an enhancer and necessary processing information sites, such as ribosome-binding sites, RNA splice sites, polyadenylation sites, transcriptional terminator sequences, and mRNA stabilizing sequences. Signal peptides may also be included where appropriate from secreted polypeptides of the same or related species, which allow the protein to cross and/or lodge in cell membranes or be secreted from the cell.

An appropriate promoter and other necessary vector sequences will be selected so as to be functional in the host. Examples of workable combinations of cell lines and expression vectors are described in Sambrook *et al.* (1989) *vide infra*; Ausubel *et al.* (Eds.) (1995) *Current Protocols in Molecular Biology*, Greene Publishing and Wiley Interscience, New York; and Metzger *et al.* (1988) *Nature*, **334**: 31-36. Many useful vectors for expression in bacteria, yeast, fungal, mammalian, insect, plant or other cells are well known in the art and may be obtained such vendors as Stratagene, New England Biolabs, Promega Biotech, and others. In addition, the construct may be joined to an amplifiable gene (e.g., DHFR) so that multiple copies of the gene may be made. For appropriate enhancer and other expression control sequences, see also *Enhancers and Eukaryotic Gene Expression*, Cold Spring Harbor Press, N.Y. (1983). While such expression vectors

may replicate autonomously, they may less preferably replicate by being inserted into the genome of the host cell.

Expression and cloning vectors will likely contain a selectable marker, that is, a gene encoding a protein necessary for the survival or growth of a host cell transformed with the vector. Although such a marker gene may be carried on another polynucleotide sequence co-introduced into the host cell, it is most often contained on the cloning vector. Only those host cells into which the marker gene has been introduced will survive and/or grow under selective conditions. Typical selection genes encode proteins that (a) confer resistance to antibiotics or other toxic substances, e.g., ampicillin, neomycin, methotrexate, etc.; (b) complement auxotrophic deficiencies; or (c) supply critical nutrients not available from complex media. The choice of the proper selectable marker will depend on the host cell; appropriate markers for different hosts are known in the art.

Recombinant host cells, in the present context, are those which have been genetically modified to contain an isolated DNA molecule of the instant invention. The DNA can be introduced by any means known to the art which is appropriate for the particular type of cell, including without limitation, transformation, lipofection or electroporation.

It is recognized by those skilled in the art that the DNA sequences may vary due to the degeneracy of the genetic code and codon usage. All DNA sequences which code for the polypeptide or protein of interest are included in this invention.

Additionally, it will be recognized by those skilled in the art that allelic variations may occur in the DNA sequences which will not significantly change activity of the amino acid sequences of the peptides which the DNA sequences encode. All such equivalent DNA sequences are included within the scope of this invention and the definition of the regulated promoter region. The skilled artisan will understand that the sequence of the exemplified sequence can be used to identify and isolate additional, nonexemplified nucleotide sequences which are functionally equivalent to the sequences given.

Mutational, insertional, and deletional variants of the disclosed nucleotide sequences can be readily prepared by methods which are well known to those skilled in the art. These variants can be used in the same manner as the exemplified primer sequences so long as the variants have substantial sequence homology with the original sequence. As used herein, substantial sequence homology refers to homology which is sufficient to enable the variant polynucleotide to function in the same capacity as the polynucleotide from which the probe was derived. Preferably, this homology is greater than 80%, more preferably, this homology is greater than 85%, even more preferably this homology is greater than 90%, and most preferably, this homology is greater than 95%. The degree of homology or identity needed for the variant to function in its intended capacity depends upon the intended use of the sequence. It is well within the skill of a person trained in this art to make mutational, insertional, and deletional mutations which are equivalent in function or are designed to improve the function of the sequence or otherwise provide a methodological advantage.

Polymerase Chain Reaction (PCR) is a repetitive, enzymatic, primed synthesis of a nucleic acid sequence. This procedure is well known and commonly used by those skilled in this art [see Mullis, U.S. Patent Nos. 4,683,195, 4,683,202, and 4,800,159; Saiki *et al.* (1985) *Science* 230:1350-1354]. PCR is based on the enzymatic amplification of a DNA fragment of interest that is flanked by two oligonucleotide primers that hybridize to opposite strands of the target sequence. The primers are oriented with the 3' ends pointing towards each other. Repeated cycles of heat denaturation of the template, annealing of the primers to their complementary sequences, and extension of the annealed primers with a DNA polymerase result in the amplification of the segment defined by the 5' ends of the PCR primers. Since the extension product of each primer can serve as a template for the other primer, each cycle essentially doubles the amount of DNA template produced in the previous cycle. This results in the exponential accumulation of the specific target fragment, up to several million-fold in a few hours. By using a thermostable DNA polymerase such as the *Taq* polymerase, which is isolated from the thermophilic bacterium *Thermus aquaticus*, the amplification process can be completely automated. Other enzymes which can be used are known to those skilled in the art.

It is well known in the art that the polynucleotide sequences of the present invention can be truncated and/or mutated such that certain of the resulting fragments and/or mutants of the original full-length sequence can retain the desired characteristics of the full-length sequence. A wide variety of restriction enzymes which are suitable for generating fragments from larger nucleic acid molecules are well known. In addition, it is well known that *Bal*31 exonuclease can be conveniently used for time-controlled limited digestion of DNA. See, for example, Maniatis (1982) *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, New York, pages 135-139, incorporated herein by reference. See also Wei *et al.* (1983 *J. Biol. Chem.* **258**:13006-13512. By use of *Bal*31 exonuclease (commonly referred to as "erase-a-base" procedures), the ordinarily skilled artisan can remove nucleotides from either or both ends of the subject nucleic acids to generate a wide spectrum of fragments which are functionally equivalent to the subject nucleotide sequences. One of ordinary skill in the art can, in this manner, generate hundreds of fragments of controlled, varying lengths from locations all along the original molecule. The ordinarily skilled artisan can routinely test or screen the generated fragments for their characteristics and determine the utility of the fragments as taught herein. It is also well known that the mutant sequences of the full length sequence, or fragments thereof, can be easily produced with site directed mutagenesis. See, for example, Larionov, O.A. and Nikiforov, V.G. (1982) *Genetika* **18**(3):349-59; Shortle, D, DiMaio, D., and Nathans, D. (1981) *Annu. Rev. Genet.* **15**:265-94; both incorporated herein by reference. The skilled artisan can routinely produce deletion-, insertion-, or substitution-type mutations and identify those resulting mutants which contain the desired characteristics of the full length wild-type sequence, or fragments thereof, i.e., those which retain promoter activity and also provide transcription of downstream sequence.

Following the teachings herein and using knowledge and techniques well known in the art, the skilled worker will be able to make a large number of operative embodiments having equivalent DNA sequences to those listed herein without the expense of undue experimentation.

As used herein percent sequence identity of two nucleic acids is determined using the algorithm of Karlin and Altschul (1990) *Proc. Natl. Acad. Sci. USA*

87:2264-2268, modified as in Karlin and Altschul (1993) *Proc. Natl. Acad. Sci. USA*
90:5873-5877. Such an algorithm is incorporated into the NBLAST and XBLAST
programs of Altschul *et al.* (1990) *J. Mol. Biol.* **215**:402-410. BLAST nucleotide
searches are performed with the NBLAST program, score = 100, wordlength = 12,
5 to obtain nucleotide sequences with the desired percent sequence identity. To
obtain gapped alignments for comparison purposes, Gapped BLAST is used as
described in Altschul *et al.* (1997) *Nucl. Acids. Res.* **25**:3389-3402. When utilizing
BLAST and Gapped BLAST programs, the default parameters of the respective
programs (NBLAST and XBLAST) are used. See, for example, the National Center
10 for Biotechnology Information website on the internet.

Techniques and agents for introducing and selecting for the presence of
heterologous DNA in plant cells and/or tissue are well-known. Genetic markers
allowing for the selection of heterologous DNA in plant cells are well-known, e.g.,
15 genes carrying resistance to an antibiotic such as kanamycin, hygromycin,
gentamicin, or bleomycin. The marker allows for selection of successfully
transformed plant cells growing in the medium containing the appropriate antibiotic
because they will carry the corresponding resistance gene. In most cases the
heterologous DNA which is inserted into plant cells contains a gene which encodes
20 a selectable marker such as an antibiotic resistance marker, but this is not
mandatory. An exemplary drug resistance marker is the gene whose expression
results in kanamycin resistance, i.e., the chimeric gene containing nopaline
synthetase promoter, Tn5 neomycin phosphotransferase II and nopaline
synthetase 3' non-translated region described by Rogers *et al.*, *Methods for Plant*
25 *Molecular Biology*, A. Weissbach and H. Weissbach, eds., Academic Press, Inc.,
San Diego, CA (1988).

Techniques for genetically engineering plant cells and/or tissue with an
expression cassette comprising an inducible promoter or chimeric promoter fused
30 to a heterologous coding sequence, including possibly an antisense DNA construct
and/or a DNA construct designed to elicit double-stranded RNA-mediated gene
silencing, followed by a transcription termination sequence are to be introduced into
the plant cell or tissue by *Agrobacterium*-mediated transformation, electroporation,
microinjection, particle bombardment or other techniques known to the art. The

expression cassette advantageously further contains a marker allowing selection of the heterologous DNA in the plant cell, e.g., a gene carrying resistance to an antibiotic such as kanamycin, hygromycin, gentamicin, or bleomycin.

5 A DNA construct carrying a plant-expressible gene or other DNA of interest can be inserted into the genome of a plant by any suitable method. Such methods may involve, for example, the use of liposomes, electroporation, diffusion, particle bombardment, microinjection, gene gun, chemicals that increase free DNA uptake, e.g., calcium phosphate coprecipitation, viral vectors, and other techniques
10 practiced in the art. Suitable plant transformation vectors include those derived from a Ti plasmid of *Agrobacterium tumefaciens*, such as those disclosed by Herrera-Estrella (1983), Bevan (1983), Klee (1985) and EPO publication 120,516 (Schilperoort *et al.*). In addition to plant transformation vectors derived from the Ti or root-inducing (Ri) plasmids of *Agrobacterium*, alternative methods can be used
15 to insert the DNA constructs of this invention into plant cells.

 The choice of vector in which the DNA of interest is operatively linked depends directly, as is well known in the art, on the functional properties desired, e.g., replication, protein expression, and the host cell to be transformed, these
20 being limitations inherent in the art of constructing recombinant DNA molecules. The vector desirably includes a prokaryotic replicon, i.e., a DNA sequence having the ability to direct autonomous replication and maintenance of the recombinant DNA molecule extra-chromosomally when introduced into a prokaryotic host cell, such as a bacterial host cell. Such replicons are well known in the art. In addition,
25 preferred embodiments that include a prokaryotic replicon also include a gene whose expression confers a selective advantage, such as a drug resistance, to the bacterial host cell when introduced into those transformed cells.

 Typical bacterial drug resistance genes are those that confer resistance to
30 ampicillin or tetracycline, among other selective agents. The neomycin phosphotransferase gene has the advantage that it is expressed in eukaryotic as well as prokaryotic cells.

Those vectors that include a prokaryotic replicon also typically include convenient restriction sites for insertion of a recombinant DNA molecule of the present invention. Typical of such vector plasmids are pUC8, pUC9, pBR322, and pBR329 available from BioRad Laboratories (Richmond, CA) and pPL, pK and K223 available from Pharmacia (Piscataway, NJ), and pBLUESCRIPT and pBS available from Stratagene (La Jolla, CA). A vector of the present invention may also be a Lambda phage vector including those Lambda vectors described in Molecular Cloning: A Laboratory Manual, Second Edition, Maniatis *et al.*, eds., Cold Spring Harbor Press (1989) and the Lambda ZAP vectors available from Stratagene (La Jolla, CA). Other exemplary vectors include pCMU [Nilsson *et al.* (1989) *Cell* 58:707]. Other appropriate vectors may also be synthesized, according to known methods; for example, vectors pCMU/Kb and pCMUII used in various applications herein are modifications of pCMUIV [Nilsson, (1989) *supra*].

Typical expression vectors capable of expressing a recombinant nucleic acid sequence in plant cells and capable of directing stable integration within the host plant cell include vectors derived from the tumor-inducing (Ti) plasmid of *Agrobacterium tumefaciens* described by Rogers *et al.* (1987) *Meth. in Enzymol.* 153:253-277, and several other expression vector systems known to function in plants. See for example, Verma *et al.*, No. WO87/00551; Cocking and Davey (1987) *Science* 236:1259-1262.

A transgenic plant can be produced by any means known to the art, including but not limited to *Agrobacterium tumefaciens*-mediated DNA transfer, preferably with a disarmed T-DNA vector, electroporation, direct DNA transfer, and particle bombardment [See Davey *et al.* (1989) *Plant Mol. Biol.* 13:275; Walden and Schell (1990) *Eur. J. Biochem.* 192:563; Joersbo and Burnstedt (1991) *Physiol. Plant.* 81:256; Potrykus (1991) *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 42:205; Gasser and Fraley (1989) *Science* 244:1293; Leemans (1993) *Bio/Technology* 11:522; Beck *et al.* (1993) *Bio/Technology* 11:1524; Koziel *et al.* (1993) *Bio/Technology* 11:194; Vasil *et al.* (1993) *Bio/Technology* 11:1533 and Gelvin, S.B. (1999) *Curr. Opin. Biotech.* 9:227-232]. Techniques are well-known to the art for the introduction of DNA into monocots as well as dicots, as are the techniques for culturing such plant tissues and regenerating those tissues.

Standard techniques for cloning, DNA isolation, amplification and purification, for enzymatic reactions involving DNA ligase, DNA polymerase, restriction endonucleases and the like, and various separation techniques are those known and commonly employed by those skilled in the art. A number of standard techniques are described in Sambrook *et al.* (1989) *Molecular Cloning*, Second Edition, Cold Spring Harbor Laboratory, Plainview, New York; Maniatis *et al.* (1982) *Molecular Cloning*, Cold Spring Harbor Laboratory, Plainview, New York; Wu (ed.) (1993) *Meth. Enzymol.* **218**, Part I; Wu (ed.) (1979) *Meth. Enzymol.* **68**; Wu *et al.* (eds.) (1983) *Meth. Enzymol.* 100 and 101; Grossman and Moldave (eds.) *Meth. Enzymol.* **65**; Miller (ed.) (1972) *Experiments in Molecular Genetics*, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York; Old and Primrose (1981) *Principles of Gene Manipulation*, University of California Press, Berkley; Schleif and Wensink (1982) *Practical Methods in Molecular Biology*; Glover (ed.) (1985) *DNA Cloning* Vol. I and II, IRL Press, Oxford, UK; Hames and Higgins (eds.) (1985) *Nucleic Acid Hybridization*, IRL Press, Oxford, UK; Setlow and Hollaender (1979) *Genetic Engineering: Principles and Methods*, Vols. 1-4, Plenum Press, New York; and Ausubel *et al.* (1992) *Current Protocols in Molecular Biology*, Greene/Wiley, New York, NY. Abbreviations and nomenclature, where employed, are deemed standard in the field and commonly used in professional journals such as those cited herein.

All references cited in the present application are incorporated in their entirety herein by reference to the extent not inconsistent herewith.

References

1. Liljebjelke, K. et al., Enzymatic synthesis and purification of uridine diphosphate [¹⁴C]galacturonic acid: a substrate for pectin biosynthesis, *Anal. Biochem.* **225**:296-304 (1995).
2. Doong, R.L. et al., Cell free synthesis of pectin: identification and partial characterization of polygalacturonate 4- α -galacturonosyltransferase and its products from membrane preparations of tobacco (*Nicotiana tabacum* L. cv samsun) cell suspension cultures, *Plant Physiol.* **109**:141-152 (1995).
3. Doong, R.L. et al. Solubilization and characterization of a galacturonosyltransferase that synthesizes the pectic polysaccharide homogalacturonan, *The Plant Journal* **13**:363-374 (1998).
4. Scheller, H.V. et al., Pectin biosynthesis: a solubilized galacturonosyltransferase from tobacco catalyzes the transfer of galacturonic acid from UDP-galacturonic acid onto the non-reducing end of homogalacturonan, *Planta* **207**:512-517 (1999).
5. Mohnen, D. et al., A multi-enzyme approach to study pectin biosynthesis, *Annual Meeting of the American Society of Plant Physiology, July 24-July 28* Abst. No. **203**:65(1999).(Abstract)
6. Mohnen, D., Biosynthesis of pectins and galactomannans, in: "Comprehensive Natural Products Chemistry, Vol. 3, Carbohydrates and Their Derivatives including Tannins, Cellulose, and Related Lignins", B.M. Pinto., ed., *Elsevier*, Oxford, pp. 497-527 (1999).
7. Sterling, J. et al., The catalytic site of the pectin biosynthetic enzyme α -1,4-galacturonosyltransferase (GalAT) is located in the lumen of the Golgi, *Plant Physiol.* **127**:360-371 (2001).
8. Ridley, B.L. et al., Pectins: structure, biosynthesis, and oligogalacturonide-related signaling, *Phytochemistry* **57**:929-967 (2001).
9. Mohnen, D., Biosynthesis of pectins, in: "Pectins and their Manipulation", G.B. Seymour et al., Blackwell Publishing and CRC Press, Oxford, pp. 52-98 (2002).
10. Villemez, C.L. et al., Properties of a polygalacturonic acid-synthesizing enzyme system from *Phaseolus aureus* seedlings. *Arch. Biochem. Biophys.* **116**:446-452 (1966).
11. Kauss, H. et al., Cooperation of enzymes responsible for polymerization and methylation in pectin biosynthesis. *Z. Naturforsch.* **24**:28-33 (1969).
12. Lin, T.-Y. et al., Substrate specificity in pectin synthesis. *Biochem. Biophys. Res. Commun.* **22**:650-657 (1966).

13. Bolwell, G. P, *et al.*, Decrease of polygalacturonic acid synthase during xylem differentiation in sycamore. *Phytochemistry* **24**:699-702 (1985).
14. Takeuchi, Y. *et al.*, In vitro biosynthesis of homogalacturonan by a membrane-bound galacturonosyltransferase from epicotyls of azuki bean, *Biosci. Biotech. Biochem.* **65**:1519-1527 (2001).
15. Akita, K. *et al.*, Successive glycosyltransfer activity and enzymatic characterization of pectic polygalacturonate 4- α -galacturonosyltransferase solubilized from pollen tubes of *Petunia axillaris* using pyridylaminated oligogalacturonates as substrates, *Plant Physiol.* **130**:374-379 (2002).
16. Reithmeier, R.A.F. *et al.*, Intrinsic membrane protein structure: principles and prediction, in: "The Structure of Biological Membranes", P. Yeagle., ed., CRC Press, Boca Raton, pp. 337-393 (1992).
17. Northcote, D.H., The Golgi apparatus. *Endeavor* **30**:26-33 (1971).
18. Northcote, D.H. *et al.*, A function of the Golgi Apparatus in polysaccharide synthesis and transport in the root-cap cells of wheat, *Biochem. J.* **98**:159-167 (1966).
19. Harrism, P.J. *et al.*, Polysaccharide formation in plant golgi bodies. *Biochim. Biophys. Acta* **237**:56-64 (1971).
20. Stoddart, R.W. *et al.*, Metabolic relationships of the isolated fractions of the pectic substances of actively growing sycamore cells. *Biochem. J.* **105**:45-59 (1967).
21. Moore, P.J. *et al.*, Spatial organization of the assembly pathways of glycoproteins and complex polysaccharides in the golgi apparatus of plants. *J. Cell Biol.* **112**:589-602 (1991).
22. Staehelin, L.A. *et al.*, The plant Golgi apparatus: structure, functional organization and trafficking mechanisms, *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **46**:261-288 (1995).
23. Willats, W.G.T. *et al.*, Making and using antibody probes to study plant cell walls, *Plant Physiol. Biochem.* **38**:27-36 (2000).
24. Goubet, F. *et al.*, Subcellular localization and topology of homogalacturonan methyltransferase in suspension-cultured *Nicotiano tabacum* cells, *Planta* **209**:112-117 (1999).
25. Vannier, M.P. *et al.*, Localization of methyltransferase activities throughout the endomembrane complex system of flax (*Linum usitatissimum* L) hypocotyls, *Biochem. J.* **286**:863-868 (1992).
26. Bourlard, T. *et al.*, Various pectin methyltransferase activities with affinity for low and highly methylated pectins, *Plant Cell Physiol.* **38**:259-267 (1997).

27. O'Neill, M. *et al.*, The pectic polysaccharides of primary cell walls, in: "Methods in Plant Biochemistry, Volume 2", P.M. Dey., ed., Academic Press, London, pp. 415-441 (1990).
28. Lau, J.M., *et al.*, Structure of the backbone of rhamnogalacturonan I, a pectic polysaccharide in the primary cell walls of plants, *Carbohydr. Res.* **137**:111-125 (1985).
29. Eda, S. *et al.*, A pectic polysaccharide from cell walls of tobacco (*Nicotiana tabacum*) mesophyll, *Carbohydr. Res.* **158**:205-216 (1986).
30. Carpita, N.C. *et al.*, Structural models of primary cell walls in flowering plants: consistency of molecular structure with the physical properties of the walls during growth, *Plant J.* **3**:1-30 (1993).
31. O'Neill, M.A. *et al.*, Rhamnogalacturonan-II, a pectic polysaccharide in the walls of growing plant cell, forms a dimer that is covalently cross-linked by a borate ester - in vitro conditions for the formation and hydrolysis of the dimer, *J. Biol. Chem.* **272**:3869(1997).
32. Schols, H.A. *et al.*, A xylogalacturonan subunit present in the modified hairy regions of apple pectin, *Carbohydr. Res.* **279**:265-279 (1995).
33. Kikuchi, A. *et al.*, A xylogalacturonan whose level is dependent on the size of cell clusters is present in the pectin from cultured carrot cells, *Planta* **200**:369-372 (1996).
34. Yu, L. *et al.*, Partial characterization of xylogalacturonans from cell walls of ripe watermelon fruit: Inhibition of endopolygalacturonase activity by xylosylation, in: "Pectins and Pectinases", J. Visser *et al.*, Elsevier, Amsterdam, pp. 79-88 (1996).
35. Aspinall, G.O., Chemistry of cell wall polysaccharides. in: "The Biochemistry of Plants, Vol.3.", J. Preiss., ed., Academic Press, New York, pp. 473-500 (1980).
36. Watson, R.R. *et al.*, Chemistry and biochemistry of apiose, *Adv. Carbohydr. Chem. Biochem.* **31**:135-184 (1975).
37. Hart, D.A. *et al.*, Isolation and partial characterization of apiogalacturonans from the cell wall of *Lemna minor*, *Biochem. J.* **116**:569-579 (1970).
38. Schols, H.A. *et al.*, Structural features of hairy regions of pectins isolated from apple juice produced by the liquefaction process, *Carbohydr. Res.* **206**:117-129 (1990).
39. Mohnen, D. *et al.*, Cell free synthesis of the pectic polysaccharide homogalacturonan, in: "Pectins and Pectinases", J. Visser *et al.*, Elsevier Science B.V. Amsterdam, pp. 109-126 (1996).

40. Willats, W.G.T. *et al.*, Pectin: cell biology and prospects for functional analysis, *Plant Mol. Biol.* **47**:9-27 (2001).
41. An, J. *et al.*, Isolation and structural characterization of alpha-D-glucosyluronic acid and 4-O-methyl alpha-D-glucosyluronic acid-containing oligosaccharides from the cell-wall pectic polysaccharide, rhamnogalacturonan I, *Carbohydr. Res.* **252**:235-243 (1994).
42. An, J. *et al.*, Isolation and structural characterization of endo-rhamnogalacturonase-generated fragments of the backbone of rhamnogalacturonan I, *Carbohydr. Res.* **264**:83-96 (1994).
43. O'Neill, M.A. *et al.*, Requirement of borate cross-linking of cell wall rhamnogalacturonan II for *Arabidopsis* growth, *Science* **294**:846-849 (2001).
44. Atkinson, R.G. *et al.*, Overexpression of polygalacturonase in transgenic apple trees leads to a range of novel phenotypes involving changes in cell adhesion, *Plant Physiol.* **129**:122-133 (2002).
45. Mohnen, D. *et al.*, Cell wall carbohydrates as signals in plants, *Sem. Cell Biol.* **4**:93-102 (1993).
46. Côté, F. *et al.*, Oligosaccharide elicitors in host-pathogen interactions generation, perception, and signal transduction, in: "Plant-Microbe Interactions", B.B. Biswas *et al.*, Plenum Press, New York, pp. 385-432 (1998).
47. Mollet, J.-C. *et al.*, A lily stylar pectin is necessary for pollen tube adhesion to an in vitro stylar matrix, *Plant Cell* **12**:1737-1749 (2000).
48. Western, T.L. *et al.*, Isolation and characterization of mutants defective in seed coat mucilage secretory cell development in *Arabidopsis*, *Plant Physiol.* **127**:998-1011 (2001).
49. Willats, W.G.T. *et al.*, In-situ analysis of pectic polysaccharides in seed mucilage and at the root surface of *Arabidopsis thaliana*, *Planta* **213**:37-44 (2001).
50. González-Carranza, Z.H. *et al.*, Temporal and spatial expression of a polygalacturonase during leaf and flower abscission in oilseed rape and *Arabidopsis*, *Plant Physiol.* **128**:534-543 (2002).
51. Brown, K., Xylem may direct water where it's needed, *Science* **291**:571-572 (2001).
52. Fry, S. *et al.*, Oligosaccharides as Signals and Substrates in the Plant Cell Wall, *Plant Physiol.* **103**:1-5 (1993).
53. Shibuya, N. *et al.*, Oligosaccharide signalling for defence responses in plant, *Physiol. Mol. Plant Pathol.* **59**:223-233 (2001).

54. Skjot, M. *et al.*, Direct interference with rhamnogalacturonan I biosynthesis in Golgi vesicles, *Plant Physiol.* **129**:95-102 (2002).
55. Bouton, S. *et al.*, QUASIMODO1 encodes a putative membrane-bound glycosyltransferase required for normal pectin synthesis and cell adhesion in *Arabidopsis*, *Plant Cell* **14**:2577-2590 (2002).
56. Edwards, M.E. *et al.*, Molecular characterisation of a membrane-bound galactosyltransferase of plant cell wall matrix polysaccharide biosynthesis, *Plant J.* **19**:691-697 (1999).
57. Perrin, R.M. *et al.*, Xyloglucan fucosyltransferase, an enzyme involved in plant cell wall biosynthesis, *Science* **284**:1976-1979 (1999).
58. Faik, A. *et al.*, An *Arabidopsis* gene encoding an alpha-xylosyltransferase involved in xyloglucan biosynthesis, *Proc. Natl. Acad. Sci. USA* **99**:7797-7802 (2002).
59. Delmer, D.P., A hot mutant for cellulose synthesis, *Trends in Plant Science* **3**:164 (1998).
60. Orellana, A. *et al.*, Enzymatic synthesis and purification of [³H] uridine diphosphate galacturonic acid for use in studying Golgi-localized transporters, *Analytical Biochemistry* **272**:224-231 (1999).
61. Crombie, H.J. *et al.*, A homogalacturonan synthase from mung bean hypocotyls, Cell Wall '01 - 9th International Cell Wall Meeting, September 2-7, 2001 Toulouse, France 131(2001).(Abstract)
62. Cumming, C.M. *et al.*, A galacturonyltransferase involved in pectin biosynthesis. in: "Cell Walls '86. Proceedings of the Fourth Cell Wall Meeting. Paris - September 10-12, 1986", B. Vian *et al.*, Université Pierre et Marie Curie - Ecole Normale Supérieure. Paris, pp. 360-363 (1986).
63. Sterling, J.D. *et al.*, Development of a filter assay for measuring homogalacturonan:alpha1,4-galacturonosyltransferase activity, (in preparation)
64. Williams, N., Rain forest fragments fare poorly, *Science* **278**:1016(1997).
65. Pagès, S. *et al.*, Changing a single amino acid residue switches processive and non-processive behavior of *Aspergillus niger* endopolygalacturonase I and II, *J. Biol. Chem.* **276**:33652-33656 (2001).
66. Campbell, J.A. *et al.*, A classification of nucleotide-diphospho-sugar glycosyltransferases based on amino acid sequence similarities, *Biochem. J.* **326**:929-942 (1997).
67. Henrissat, B. *et al.*, Glycoside hydrolases and glycosyltransferases. Families, modules, and implications for genomics. *Plant Physiol* **124**:1515-1519 (2000).

68. Jones, D.T., GenTHREADER: An Efficient and Reliable Protein Fold Recognition Method for Genomic Sequences, *J. Mol. Biol.* **287**:797-815 (1999).
69. McGuffin, L.J. *et al.*, The PSIPRED protein structure prediction server, *Bioinform. Applic. Note.* **16**:404-405 (2000).
70. Persson, K. *et al.*, Crystal structure of the retaining galactosyltransferase LgtC from *Neisseria meningitidis* in complex with donor and acceptor sugar analogs, *Nature Structural Biology* **8**:166-175 (2001).
71. Price, N.J. *et al.*, Plant glycosyltransferases, *Curr. Opin. Plant Biol.* **2001**:219-224 (2001).
72. Charnock, S.J. *et al.*, Three-dimensional structures of UDP-sugar glycosyltransferases illuminate the biosynthesis of plant polysaccharides, *Plant Physiol.* **125**:527-531 (2001).
73. Ünligil, U.M. *et al.*, Glycosyltransferase structure and mechanism, *Current Opinion in Structural Biology* **10**:510-517 (2000).
74. Boix, E. *et al.*, Structural basis of ordered binding of donor and acceptor substrates to the retaining glycosyltransferase, alpha-1,3-galactosyltransferase, *J. Biol. Chem.* **277**:28310-28318 (2002).
75. Cabral, C.M. *et al.*, Organizational diversity among distinct glycoprotein endoplasmic reticulum-associated degradation programs, *Mol. Biol. Cell* **13**:2639-2650 (2002).
76. Mallisard, M. *et al.*, Expression of functional soluble forms of human alpha-1,4-galactosyltransferase I, alpha-2,6-sialyltransferase, and alpha-2,6-sialyltransferase, and alpha-1,3-fucosyltransferase VI in the methylotrophic yeast *Pichia pastoris*, *Biochem. Biophys. Res. Commun.* **267**:169-173 (2000).
77. Romero, P.A. *et al.*, KTR1P is an alpha-1,2-mannosyltransferase of *Saccharomyces cerevisiae* comparison of the enzymatic properties of soluble recombinant KTR1P and KRE2P/MNT1P produced in *Pichia pastoris*, *Biochemical Journal* **321**:289-295 (1997).
78. Hochstrasser, U. *et al.*, Expression of a functional barley sucrose-fructan 6-fructosyltransferase in the methylotrophic yeast *Pichia pastoris*, *Febs Letters* **440**:356-360 (1998).
79. Gallet, P.F. *et al.*, Heterologous expression of an engineered truncated form of human Lewis fucosyltransferase (Fuc-TIII) by the methylotrophic yeast *Pichia pastoris*, *Glycobiology* **8**:919-925 (1998).
80. Moreman, K.W. *et al.*, Topology of mannosidase II in rat liver membrane and release of the catalytic domain by selective proteolysis, *Jr Biol. Chem* **23**:10945-10951 (1986).

81. Merkle, R.K. *et al.*, Cloning, expression, purification, and characterization of the murine lysosomal acid alpha-mannosidase, *Biochim. Biophys. Acta* **1336**:132-146 (1997).
82. Liao, Y.F. *et al.*, Cloning, expression, purification, and characterization of the human broad-specificity lysosomal acid alpha-mannosidase, *J. Biol. Chem.* **271**:28348-28358 (1996).
83. Bar-Peled, M. *et al.*, UDP-rhamnose:flavanone-7-O-glucoside-2"-O-rhamnosyltransferase. Purification and characterization of an enzyme catalyzing the production of bitter compounds in citrus, *J. Biol. Chem.* **266**:20953-20959 (1991).
84. Basu, S.S. *et al.*, A facile enzymatic synthesis of uridine diphospho-[14C]galacturonic acid, *Anal. Biochem.* **280**:173-177 (2000).
85. Quigley, H.F. *et al.*, A non-radioactive gel electrophoresis assay for homogalacturonan alpha-1,4-galacturonosyltransferase, (in preparation).
86. Bao, M. *et al.*, Bovine UDP-N-acetylglucosamine:lysosomal-enzyme N-acetylglucosamine-1-phosphotransferase. I. Purification and subunit structure, *J. Biol. Chem.* **271**:31437-31445 (1996).
87. Briand, J.P. *et al.*, Application and limitations of the multiple antigen peptide (MAP) system in the production and evaluation of anti-peptide and anti-protein antibodies, *J. Immunol. Methods* **156**:255-265 (1992).
88. Ziehl, V. *et al.*, Polyclonal antibodies directed against synthetic N-terminus of fungal endopolygalacturonase recognizes the native protein and cross-reacts with endopolygalacturonase of a different fungal species, Abstract, Pectins and Pectinases meeting, Wageningen, Netherlands, (1995)
89. Harlow, E. *et al.*, Antibodies: A laboratory manual, Cold Spring Harbor Press, Cold Spring Harbor, NY, (1988).
90. Mohnen, D. *et al.*, Hormonal regulation of alpha1,3-glucanase messenger RNA levels in cultured tobacco tissues. *EMBO J.* **4**:1631-1635 (1985).
91. Zhang G.F. *et al.*, Functional compartmentation of the golgi apparatus of plant cells; Immunocytochemical analysis of high-pressure frozen- and freeze-substituted sycamore maple suspension culture cells, *Plant Physiol.* **99**:1070-1083 (1992).
92. Colley, K.J., Golgi localization of glycosyltransferases: more questions than answers, *Glycobiology* **7**:1-13 (1997).
93. Opat, A.S. *et al.*, Trafficking and localization of resident Golgi glycosylation enzymes, *Biochimie* **83**:763-773 (2001).

94. Dirnberger, D. *et al.*, The Golgi localization of *Arabidopsis thaliana* B1,2-xylosyltransferase in plant cells is dependent on its cytoplasmic and transmembrane sequences, *Plant Mol. Biol.* **50**:273-281 (2002).
95. Munro, S., An investigation of the role of transmembrane domains in golgi protein retention, *The EMBO Journal* **14**:4695-4704 (1995).
96. Nilsson, T. *et al.*, Kin recognition. A model for the retention of Golgi enzymes. *FEBS Lett.* **330**:1-4 (1993).
97. Freshour, G. *et al.*, Developmental and tissue-specific structural alterations of the cell-wall polysaccharides of *Arabidopsis thaliana* Roots, *Plant Physiol.* **110**:1413-1429 (1996).
98. Samuels, A.L. *et al.*, Cytokinesis in tobacco BY-2 and root tip cells: a new model of cell plate formation in higher plants, *J. Cell Biol.* **130**:(1995).
99. Lam, B.C.-H. *et al.*, Role of SH3 Domain-Containing Proteins in Clathrin-Mediated Vesicle Trafficking in *Arabidopsis*, *Plant Cell* **13**:2499-2512 (2001).
100. Chuang, C.-F. *et al.*, Specific and heritable genetic interference by double-stranded RNA in *arabidopsis thaliana*, *Proc. Natl. Acad. Sci. USA* **97**:4985-4990 (2000).
101. Bent, A.F. *et al.*, *RPS2* of *Arabidopsis thaliana*: A Leucine-Rich Repeat Class of Plant Disease Resistance Genes, *Science* **265**:1856-1860 (1994).
102. Bieberich, E. *et al.*, Regulation of Ganglioside Biosynthesis by Enzyme Complex Formation of Glycosyltransferases, *Biochem.* **41**:11479-11487 (2002).
103. Ridley, B.L. *et al.*, A method for biotin labeling of biologically active oligogalacturonides using a chemically stable hydrazide linkage, *Anal. Biochem.* **249**:10-19 (1997).
104. Guillaumie, F. *et al.*, Solid-phase biosynthesis and MALDI-TOF mass spectrometry analysis of pectic oligogalacturonides: a new tool to monitor the extension of a homogalacturonan chain, *Carbohydr. Res.* (2002).
105. Yamada, H. *et al.*, Structural Characterization and Antitumor Activity of a Pectic Polysaccharide from the Roots of *Angelica acutiloba*. *planta medica* **56**:182-186, (1990).
106. Olano-Martin, E. *et al.*, Pectin and pectic-oligosaccharides induce apoptosis in *in vitro* human colonic adenocarcinoma cells. *Anticancer research* **23**:341-346, (2003.).
107. Avivi-Green, C. *et al.*, Pectin-enriched diet affects distribution and expression of apoptosis-cascade proteins in colonic crypts of dimethylhydrazine-treated rats. *Int J Mol Med* **6**:689-698 (2000a).

108. Avivi-Green, C. *et al.*, Apoptosis cascade proteins are regulated in vivo by high intracolonic butyrate concentration: correlation with colon cancer inhibition. *Oncol Res* **12**:83-95 (2000b).
109. Ohno, K. *et al.*, Inhibitory effect of apple pectin and culture condensate of *Bifidobacterium longum* on colorectal tumors induced by 1,2-dimethylhydrazine in transgenic mice harboring human prototype c-Ha-ras genes. *Exp Anim* **49**:305-307 (2000).
110. Davidson, L.A., *et al.*, Morphodensitometric analysis of protein kinase C β expression in rat colon: modulation by diet and relation to in situ cell proliferation and apoptosis. *Carcinogenesis* **21**:1513-1519 (2000).
111. Fernandez, M.L., Citrus pectin and cholesterol interact to regulate hepatic cholesterol homeostasis and lipoprotein metabolism: a dose-response study in guinea pigs. *Am J Clin Nutr* **59**: 869-878 (1994).
112. Behall, K. *et al.*, In: ML Fishman and JJ Jen, eds, Chemistry and Function of Pectins, American Chemical Society, Washington, D.C. pp 248-265 (1986).
113. Levitt, N.S. *et al.*, The effect of dietary fiber on glucose and hormone responses to a mixed meal in normal subjects and in diabetic subjects with and without autonomic neuropathy. *Diabetes Care* **3**: 505-519 (1980).
114. Hayashi, A. *et al.*, Effects of daily oral administration of quercetin chalcone and modified citrus pectin on implanted colon-25 tumor growth in bulb-c mice. *Altern Med Rev* **5**: S-546-552 (2000).
115. Inohara, H. *et al.*, Effects of natural complex carbohydrates (citrus pectin) on murine melanoma cell properties related to galectin-3 functions. *Glycoconjugates Journal* **11**: 527-532 (1994).
116. Dongowski, G, *et al.*, In: J Visser and AGJ Voragen, eds, Pectins and Pectinases, Elsevier Science B.V. Amsterdam, pp 659-666 (1996).
117. Honjo, Y. *et al.*, Expression of Cytoplasmic Galectin-3 as a Prognostic Marker in Tongue Carcinoma. *Clinical Cancer Research*. **6**(12):4635-40, (2000).
118. Honjo, Y. *et al.*, Down-Regulation of Galectin-3 Suppresses Tumorigenicity of Human Breast Carcinoma Cells. *Clinical Cancer Research*. **7**(3):661-8, (2001).
119. Yoshii, T. *et al.*, Galectin-3 maintains the transformed phenotype of thyroid papillary carcinoma cells. *Int. J. Oncol.* **18**(4):787-92, (2001).
120. Naik, H. *et al.*, Inhibition of *in vitro* tumor cell endothelial adhesion by modified citrus pectin: a pH modified natural complex carbohydrate (Meeting abstract) *Proc Annul Meet Am Assoc Cancer Res.*; **36**: 377 (1995).
121. Platt, D. *et al.*, Modulation of the lung colonization of B16-F1 melanoma cells by citrus pectin. *J Natl Cancer Inst.*; **84**(6):438-442 (1992).

122. Strum, S. *et al.*, International Conference on Diet and Prevention of Cancer (Finland). May (1999).
123. Liu, C. *et al.*, Citrus pectin: characterization and inhibitory effect on fibroblast growth factor-receptor interaction. *J Agric Food Chem* **49**: 3051-3057 (2001).
124. Rolin, C. Pectin. In: RL Whistler and JN BeMiller, eds, Industrial Gums - Polysaccharides and Their Derivatives, Third Ed. Academic Press, San Diego, pp 257-293 (1993).
125. Nangia-Makker, P. *et al.* Inhibition of human cancer cell growth and metastasis in nude mice by oral intake of modified citrus pectin. *J. Natl. Cancer Inst.* **94**: 1854-1862 (2002).
126. Dongowski, G *et al.*, Degradation of pectins with different degrees of esterification by *Bacteroides thetaiotaomicron* isolated from human gut flora. *Appl Environ Microbiol* **66**: 1321-1327 (2000).
127. Pienta, K.J. *et al.*, Inhibition of spontaneous metastasis in a rat prostate cancer model by oral administration of modified citrus pectin, *J. Natl. Cancer Inst.* **87**:348-353 (1995).
128. Zwieniecki, M.A. *et al.*, Hydrogel control of xylem hydraulic resistance in plants, *Science* **291**:1059-1062 (2001).
129. Skjot, M. *et al.*, Direct interference with rhamnogalacturonan I biosynthesis in Golgi vesicles, *Plant Physiol.* **129**:95-102 (2002).
130. Iwai, H., A pectin glucuronyltransferase gene is essential for intercellular attachment in the plant meristem, *Proc. Natl. Acad. Sci. USA* **99**:16319-16324 (2002).
131. York, W.S. *et al.*, Isolation and characterization of plant cell walls and cell wall components. *Methods Enzymol.* **118**:3-40 (1985).
132. Vidal, S. *et al.*, Structural characterization of the pectic polysaccharide rhamnogalacturonan II: evidence for the backbone location of the aceric acid-containing oligoglycosyl side chain, *Carbohydr. Res.* **326**:277-294 (2000).
133. Whitcombe, A.J. *et al.*, Structural characterization of the pectic polysacchride, Rhamnogalacturonan-II, *Carbohydr. Res.* **271**:15-29 (1995).
134. Boyes, D.C. *et al.*, Growth stage-based phenotypic analysis of arabidopsis: a model for high throughput functional genomics in plants, *Plant Cell* **13**:1499-1510 (2001).
135. Zablackis, E. *et al.*, Characterization of the cell-wall polysaccharides of *Arabidopsis thaliana* leaves, *Plant Physiol.* **107**:1129-1138 (1995).

136. Madson, M. *et al.*, The MUR3 gene of Arabidopsis encodes a xyloglucan galactosyltransferase that is evolutionarily related to animal exostosins, *Plant Cell* **15**:1662-1670 (2003).